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CHROMOSOME ENDS IN DATURA PRUINOSA

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In the present paper we are adding *D. pruinosa* to the list of those species of *Datura*, the chromosomes of which have been given numerical representation in terms of prime type 1 (*PT*1),[1] our tester race in *D. stramonium*. *D. pruinosa* is a Mexican species which belongs to the Dutra[2] section of the genus. The tester race came from Oaxaca; other races came from the adjacent state of Puebla.

By our method of examining chromosomes at the first meiotic metaphase in pollen-mother-cells, we usually detect only those changes which have occurred in the arrangements of the ends of chromosomes,[3] as a result of segmental interchange between non-homologous chromosomes. It has taken six years to complete the identification of the chromosomes of *D. pruinosa* because, with the exception of *D. leichhardtii*, it could not be crossed with any other species in either the Stramonium or Dutra sections of the genus. Before any other crosses could be made, strains of *leichhardtii* had to be developed which included in their chromosome complement the end arrangements of the four *pruinosa* chromosomes which are different from the tester race of *leichhardtii*.[4] Even with such strains, however, no cross could be made with the tester race of *stramonium* but only with extracted lines.

There were repeated instances of incompatibilities when we attempted to obtain plants which resembled *stramonium* in morphological appearance and which were homozygous for the chromosomes of the *pruinosa* interchanges. Therefore the usual method of using homozygous types had to be abandoned and all determinations made with crosses to strains that were only heterozygous for these interchanges. Fortunately the two types of offspring from each cross of the heterozygote to homozygous *PT* testers could be distinguished cytologically because of long familiarity with the sizes and other peculiarities of the chromosomes in the various

PT tester races. These incompatibilities in crosses probably are a consequence of structural changes in the chromosomes which cannot be detected by our method of examining the meiotic chromosomes at *MI* and possibly also of gene mutations that have occurred in the different species. There were fewer barriers between *pruinosa* and other species of the same section than between it and *stramonium* which belongs to a different section of the *Datura* genus.

A cross was made between the tester race of *D. leichhardtii* and that of *D. pruinosa*. The 24 chromosomes in the hybrid were arranged as a $\odot^5 4 + \odot 4 + 8$ bivalents at the first meiotic metaphase in *PMC*. Therefore the tester races of these two species differ by two separate interchanges each of which consists of an interchange between two non-homologous chromosomes. Eight of the chromosomes of *pruinosa* have the same end arrangements as eight of the chromosomes of the *leichhardtii* tester race.

This hybrid was back-crossed to the *leichhardtii* tester. Among the offspring, there were plants which also showed $\odot 4 + \odot 4$. This backcrossing and recovery of the two circles was repeated twice more. By this time the plants resembled *leichhardtii* in external morphology. Such a plant when selfed gave among its offspring some plants that showed only 12 bivalents at *MI*. A backcross to *leichhardtii* was needed to distinguish between those bivalent plants which were homozygous for the four *leichhardtii* chromosomes and those homozygous for the four *pruinosa* chromosomes which are involved in the two $\odot 4$ configurations. The former showed only bivalents in the backcross hybrid, the latter showed $\odot 4 + \odot 4$.

Two strains of *leichhardtii*, differing slightly in external appearance, but both homozygous for the end arrangements of these four *pruinosa* chromosomes, were obtained by this method. They were continued by selfing and were used *in place of* the tester race of *pruinosa* in making crosses. This method of backcrossing apparently removed some barriers to crossability so that viable hybrids were obtained with *D. inoxia* and *D. meteloides*. The hybrid with *inoxia* type 1 showed a $\odot 8 + 8$ bivalents; the hybrid with *inoxia* type 2 showed a $\odot 8 + \odot 4 + 6$ bivalents. The hybrid with the tester race of *meteloides* showed a $\odot 8 + \odot 4 + \odot 4 + 4$ bivalents. At this time also a cross with *leichhardtii* type 2 was made. The hybrid showed a $\odot 4 + 10$ bivalents. This single interchange indicates that the end arrangements of only two *leichhardtii* type 2 chromosomes are different from those of *pruinosa*.

No viable hybrid could be obtained with either of these extracted lines of *leichhardtii* when they were crossed to the tester race of *stramonium*. Fortunately we had previously developed strains of *leichhardtii* which included in their chromosome complement the end arrangements of the four *stramonium* chromosomes which are different from the tester race of

leichhardtii.[4, 6] A successful cross could be made by using *in place of* the tester race of *stramonium* such a strain of *leichhardtii*. That is, the compatible cross was between two extracted lines of *leichhardtii*, one from a previous *leichhardtii-pruinosa* cross and the other from a previous *leichhardtii-stramonium* cross. This indirect approach, using *leichhardtii* as the bridging species, made possible a comparison of the end arrangements of the *pruinosa* chromosomes with those of our standard *PT*1 of *stramonium*. The hybrid showed a ⊙ 4 + ⊙ 4 + ⊙ 6 + 5 bivalents. Usually the ⊙ 6 was broken leaving a chain of 6 instead of the ⊙ 6, as shown in figure 1. It was concluded that only five of the chromosomes of *pruinosa* have the same end arrangements as those of *PT*1. The other seven chromosomes are different. In terms of *PT*1, two chromosomes are involved in each of two interchanges and three in the third. In order to identify these seven chromosomes, a study was made of the chromosome arrangements in crosses to selected *PT*'s of *stramonium* and chromosomal types of other species.

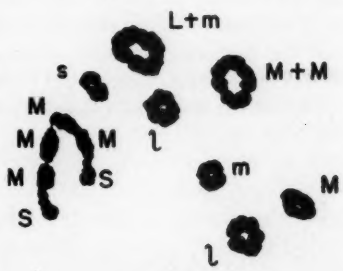

FIGURE 1

Arrangement of 24 chromosomes in extracted *pruinosa* × extracted *stramonium* hybrid as ⊙ 4 + ⊙ 4 + chain 6 + 5 bivalents. The size of each chromosome in the chain (broken circle) is given. Otherwise sizes are indicated for pairs of chromosomes in bivalents and in two circles.

This F_1 hybrid was backcrossed to another extracted line of *leichhardtii* carrying the *PT*1 chromosomes[7] with the object of introducing *stramonium* genes and consequently of building up strains more like *stramonium*. From the offspring a plant was selected which showed ⊙ 4 + ⊙ 4 + ⊙ 6. This also was backcrossed to an extracted line of *leichhardtii* which carried the same *PT*1 chromosomes but which was nearer *stramonium* in appearance. Some of the offspring from this cross showed only one or two configurations. With them it was decided to study each of the interchanges separately and since these plants now showed more resemblance to *stramonium*, attempts were made to make crosses directly to *stramonium PT* testers.[1]

That two of the *pruinosa* chromosomes are **1·18** and **2·17**[8] was proved in the following manner. From the self of a plant which showed a ⊙ 4 + ⊙ 4 there was obtained a plant which showed the same two circles of four. These were cytologically distinguishable. One circle seemed to consist of four *M*-sized chromosomes.[1, 9] The other looked like the heterozygous *PT*2 configuration (1·2–**2·17**–17·18–**18·1**) judging by the sizes of the chromosomes (L, l, m, m). That two of the chromosomes in

the circle really were 1·2 and 17·18 was known because of previous backcrosses to *leichhardtii* strains homozygous for *PT*1 chromosomes. Since the plant resembled *stramonium* in appearance, a direct cross to homozygous *PT*2 of *stramonium* was made. Proof that the other two chromosomes in this circle were **1·18** and **2·17** was given when one of the offspring of this cross to homozygous *PT*2 showed only bivalents. It may be diagrammed as follows:

⊙ 4 gamete *a* (1·2 + 17·18) × *PT*2 gamete (**1·18** + **2·17**) = ⊙ 4
" *b* (**1·18** + **2·17**) × " " " = bivalents.

Only because the ⊙ 4 plant was producing **1·18, 2·17** gametes could one find among the offspring of this cross to homozygous *PT*2 a plant showing all bivalents. These two chromosomes must have come from *pruinosa*.

That two of the *pruinosa* chromosomes are **9·14** and **10·13** was proved in the following manner. From a preliminary cross between the F_1 hybrid and homozygous *PT*7 (**9·10**^{20}, **19·20**^{10}) there was obtained a plant which showed a configuration of six as follows:

x·*y*–*y*·**10**$^{10-10}$**20·19**
| | | |
x·9–9·10$^{20-20}$**20·19**

This indicated that the 9·10 chromosome is involved in the *pruinosa* interchange as well as in *PT*7.[10]

A cross was then made between a plant which showed the circle of four *M*-sized chromosomes (not heterozygous for *PT*2) and homozygous *PT*11 (**11·13, 12·17, 14·18**). This ⊙ 4 plant produced two kinds of viable gametes, those with *PT*1 chromosomes (because of the previous backcrosses to *leichhardtii* strains homozygous for *PT*1) and those with *pruinosa* chromosomes. The *PT*1 type of gamete crossed to *PT*11 would give a ⊙ 6. If one of the chromosomes in the *pruinosa* interchange were the same as one in the *PT*11 interchange, a ⊙ 8 would be expected. Actually among the offspring there was a plant which showed a ⊙ 8. Familiarity with the sizes and other morphological peculiarities of the chromosomes, especially the interchanged chromosomes of *PT*11, permitted the following representation of the ⊙ 8:

11·12–**12·17**–17·18–**18·14**–**14·a**–*a*·*b*–**b·13**–**13·11**

That the *a*·*b* chromosome is the 9·10 chromosome had been indicated by the cross to *PT*7 and was verified by a cross to homozygous *PT*23 (**2·1·9, 10**·). Among the offspring was a plant which showed a chain of 6 as follows:

1·2–**2·1·9–9·x**–*x*·*y*–*y*·**10–10**·

The ⊙ 8 obtained from the cross with *PT*11 indicated that one of the two *M*-sized chromosomes, 11·12 or 13·14, is involved in the *pruinosa* interchange. That this is not the 11·12 chromosome was learned from crosses with *PT*3 (**11·21, 12·22**) and *PT*34 (**11·12**^{24}, **23·24**^{12}). Therefore the chromosome represented as $x \cdot y$ in the offspring from crosses with *PT*7 and *PT*23 is the 13·14 chromosome.

In order to determine the end arrangements of these two *pruinosa* chromosomes, crosses were made with those *PT*'s in our collection which involve both the 9·10 and 13·14 chromosomes. From a cross with homozygous *PT*62 (**9·14, 10·13**) there was obtained a plant which showed only bivalents. Therefore the chromosomes in this *pruinosa* interchange are **9·14** and **10·13**.

The three remaining unknown *pruinosa* chromosomes, which take part in the ⊙ 6, were determined to be **11·16, 12·22** and **15·21** in the following manner.

From a preliminary cross between the F_1 hybrid and homozygous *PT*4 (**3·21, 4·22**) there was obtained a plant which showed a ⊙ 8. This indicated that one of the chromosomes involved in the *PT*4 interchange is the same as one in the *pruinosa* interchange. From a study of size differences, it was surmised that this is the 21·22 chromosome of *PT*1.

A plant showing the ⊙ 6 was heterozygous for this *pruinosa* interchange and was expected to produce two kinds of viable gametes, those with *PT*1 chromosomes (because of the previous backcrosses to *leichhardtii* strains homozygous for *PT*1 chromosomes) and those with the three *pruinosa* chromosomes. This plant was crossed to a homozygous *PT*3 (**11·21, 12·22**) line extracted from the *quercifolia* tester race.[6, 11] All offspring showed a ⊙ 4 but two kinds could be distinguished cytologically. The *SMMS* circle was heterozygous *PT*3 and was discarded. The other circle consisted of four *M*-sized chromosomes and was the product of the union of the three *pruinosa* chromosomes and the chromosomes from *PT*3. Since only four chromosomes were in the circle, two conclusions could be drawn: that both the 11·12 and 21·22 chromosomes of *PT*1 are involved in the *pruinosa* interchange and that one of the *PT*3 chromosomes was identical, in so far as end arrangements are concerned, with one of the *pruinosa* chromosomes (either **11·21** or **12·22**). These two chromosomes had formed a bivalent. Examination of the bivalents showed no size inequalities between the two members of each bivalent.

A plant showing a ⊙ 6 was also crossed to homozygous *PT*91 (**11·22, 12·21**). The offspring that were merely heterozygous *PT*91 could be discarded because they showed only a ⊙ 4. Others showed a ⊙ 6. This ⊙ 6 indicated that, although both the 11·12 and 21·22 chromosomes of *PT*1 are involved in the *pruinosa* interchange, no *pruinosa* chromosome can be represented as either **11·22** or **12·21**.

The tester race of *D. discolor*[12] has the following interchanged chromosomes: **1·11, 2·17, 12·22, 15·21, 16·18**. A cross was made between this tester race and a plant having the ⊙ 6 (heterozygous *pruinosa* interchange). As mentioned above, this ⊙ 6 plant produced two kinds of viable gametes, those with *PT*1 chromosomes and those with *pruinosa* chromosomes. Offspring from the union of the former and *discolor* gave a ⊙ 10 and were discarded. Some offspring showed a ⊙ 6. They came from the union of a *discolor* gamete and the three *pruinosa* chromosomes. This meant that two of the *discolor* chromosomes have the same end arrangements as two of *pruinosa*. The **2·17** was ruled out because it was known to be in another interchange (previously described). The **1·11** and **16·18** chromosomes were ruled out because of the fact that the **1·18** chromosome also had been identified in the same interchange with **2·17**. Therefore the two chromosomes had to be **12·22** and **15·21**. This cross to *discolor* therefore showed that it is the **12·22** chromosome of *pruinosa* which is the same as one of the *PT*3 chromosomes, and *not* the **11·21** chromosome of *PT*3.

It was also noticed among the offspring of the *discolor* × *pruinosa* chromosome cross that one bivalent consisted of an unequal pair of chromosomes. In the cross to *PT*3 no unequal-sized pair had been seen. Therefore the **12·22** chromosome of *pruinosa* has the same length as the **12·22** chromosome of *PT*3. However, it is known that the **12·22** chromosome of *discolor*[12] is longer than the **12·22** chromosome of *PT*3. Therefore the unequal pair of chromosomes seen in the cross to *discolor* must be the $(\mathbf{12\cdot22})_2$ pair.

If two of the *pruinosa* chromosomes are **12·22** and **15·21**, then the third one must be **11·16**. In order to verify this conclusion and to determine the sizes of the **11·16** and **15·21** chromosomes, a further cross was made to the tester race of *D. ferox*. The latter has the following interchanged chromosomes: **1·18, 2·17, 11·21, 12·22, 7·20**^{16}**, 8·19, 15·16**^{20}. Again a plant with a ⊙ 6 (heterozygous for the *pruinosa* interchange) was selected and crossed to *ferox*. Offspring from the union of the gamete carrying the *PT*1 chromosomes and *ferox* could be recognized cytologically and discarded. Other offspring showed a ⊙ 4 (heterozygous *PT*2) plus a configuration of eight chromosomes. The latter was either a ⊙ 8 or a ⊙ 4 to which a chain of 4 was attached. The latter was examined carefully because it furnished the needed proof. It may be diagrammed as follows:

$$\begin{array}{l} \mathbf{19\cdot8}\text{–}8\text{·}7\text{–}\mathbf{7\cdot20}^{16\text{–}16}\mathbf{16\cdot11}\text{–}\mathbf{11\cdot21} \\ \qquad\qquad\quad | \qquad\quad | \qquad\quad | \\ \qquad\quad \mathbf{19\cdot20}^{20\text{–}20}\mathbf{16\cdot15}\text{–}\mathbf{15\cdot21} \end{array}$$

The junction of the ·16 and ·20 ends is marked by the intersection of the

circle and chain. There were four *M*-sized chromosomes in the circle. The **11·16** chromosome is slightly larger than the **15·21** chromosome.

In table 1 are shown the chromosomes of *D. pruinosa* in terms of *PT*1 of *stramonium*. For comparison, the latter are also shown as well as the chromosomes of the tester race of *D. leichhardtii*.

The number of interchanges by which one species differs from another depends in part upon what race is chosen as the tester race for each species, and consequently cannot be used as sole criterion for determining their phylogenetic relationship. However, similarity of certain arrangements of chromosome ends is very suggestive. The **11·16** chromosome which is singularly absent from all races and species of the Stramonium section which we have in our collection, occurs in both *pruinosa* and *leichhardtii* which belong to another section of the genus *Datura*.

TABLE 1

CHROMOSOMES OF THE TESTER RACES OF *Stramonium*, *Pruinosa* AND *Leichhardtii*

Stramonium PT1	*Pruinosa*	*Leichhardtii*
1·2	**1·18**	**1·18**
3·4	3·4	3·4
5·6	5·6	5·6
7·8	7·8	7·8
9·10	**9·14**	9·10
11·12	**11·16**	**11·16**
13·14	**13·10**	13·14
15·16	**15·21**	**15·12**
17·18	**17·2**	**17·2**
19·20	19·20	19·20
21·22	**12·22**	21·22
23·24	23·24	23·24

[1] Bergner, A. D., Satina, S., and Blakeslee, A. F., these PROCEEDINGS, **19**, 103–115 (1933).

[2] Safford, W. E., *Jour. Wash. Acad. Sci.*, **11**, 173–189 (1921).

[3] Blakeslee, A. F., *Univ. Pennsylvania Bicentennial Conf.* In Cytology, genetics, and evolution, 37–46 (1941).

[4] Blakeslee, A. F., *Carnegie Inst. Wash. Year Book*, **36**, 38–39 (1937).

[5] ⊙ signifies "circle of."

[6] Blakeslee, A. F., *Ibid.*, **40**, 220–224 (1941).

[7] For the sake of brevity "chromosomal end arrangement" will not be repeated but is to be understood whenever the word chromosome is used.

[8] Interchanged chromosomes are in boldface type.

[9] Satina, S., Bergner, A. D., and Blakeslee, A. F., *Amer. Jour. Bot.*, **28**, 383–390 (1941).

[10] Where the interchanged segments are limited to the terminal regions of chromosomes, these regions are represented by raised numerals. In satellite chromosomes only the satellites may be involved. This kind of interchange when heterozygous induces the "necktie" type of configuration.

[11] Bergner, A. D., and Blakeslee, A. F., these PROCEEDINGS, **18**, 151–159 (1932).

[12] Bergner, A. D., and Blakeslee, A. F., *Ibid.*, **21**, 369–374 (1935).

A COMPLEX INDUCED REARRANGEMENT OF DROSOPHILA CHROMOSOMES AND ITS BEARING ON THE PROBLEM OF CHROMOSOME RECOMBINATION

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Among the F_1 larval progeny of an irradiated *Drosophila melanogaster* male of the Oregon-R stock and an untreated Oregon-R female, there appeared one individual whose salivary-gland chromosomes revealed a complex rearrangement involving at least 32 breaks. The positions of 30 points of breakage were determined; the others remain of uncertain location. Treatment of the father included exposure to 4000 roentgens of x-rays, followed by near infrared radiation for a period of 144 hours. Details of these experiments will be furnished in another publication; for the present it seems adequate to note that the induction of the breaks is in all probability attributable to the x-rays, since no chromosome rearrangement has been found among a long series of controls derived from fathers exposed only to the near infrared.

The breakage points, recorded below, that were utilized in this complex rearrangement have been determined within the limits of accuracy imposed by the few nuclei available for study. They are:

1*B*, 3*C*, 5*D*, 11*F*, 13*E*, 15*F* in the *X*-chromosome;
24*D*, 33*E*, 40, 41, 42*C*, 49*F*, 51*E*, 56*B* in the second chromosome;
64*C*, 70*C*, 76*A*, 84*F*, 85*F*, 86*E*, 87*B*, 91*A*, 93*F*, 96*B*, 96*E*, 98*A*, 98*E*, 99*D*, 99*F* in the third chromosome;
102 in the fourth chromosome.

Identification of the lettered subdivisions was made from the maps drawn by C. B. Bridges and P. N. Bridges.

The complete pattern of recombination could not be determined cytologically because of the confusion of pairing in the chromocentral regions. However, the following sequences have been inferred from observations of continuity between the various displaced sections. (Points of recombination are indicated by sloping lines (/); the centromere is indicated by the symbol cm., a chromocentral region by chr.) Nine separate sequences were identified, and are listed below:

(*a*) Tip 3*R*-99*F*/99*D*-98*E*/84*F*-85*F*/99*F*-99*D*/98*A*-96*E*/96*B*-93*F*/40-33-*E*/70*C*-64*C*/56*B*-51*E*/87*B*-91*A*/15*F*-cm. of *X*

(*b*) Tip of *X*-1*B*/41 to cm. of 2*R*
(*c*) Tip of 4 to 102/86*E*-87*B*/76*A*-80?
(*d*) chr. of ?/91*A*-93*F*/40-cm. of 2*L*
(*e*) Tip of 2*R*-56*B*/42*C*-49*F*/chr. of ?
(*f*) chr. of ?/11*F*-13*E*/98*E*-98*A*/84*F*-81?
(*g*) Tip of 3*L*-64*C*/76*A*-70*C*/49*F*-51*E*/chr. of ?
(*h*) Tip of 2*L*-24*D*/3*C*-1*B*/5*D*-11*F*/15*F*-13*E*/chr. of ?
(*i*) chr. of ?/41-42*C*/33*E*-24*D*/3*C*-5*D*/85*F*-86*E*/102-cm. of 4

These sections include all of the essentially euchromatic portions of the chromosomes, except for the section 96*B* to 96*E*, which was not detected and which may be assumed to be missing. Only two of the breaks in the proximal heterochromatic regions could be determined with any degree of certainty; these were in 40 of 2*L* and in 41 of 2*R*. The parts separated by these two breaks maintained the constant pattern of recombination that is indicated above. In addition, at least two other breaks must have been produced in order to provide a complete set of chromosomes, each with a centromere, capable of surviving the many divisions leading to the formation of the fully developed larva. Such a viable complement could be realized if breaks had occurred also in divisions 80 and 81 to give the following sequences:

Tip of 3*R* to cm. of *X* (as detailed in (*a*) above)
Tip *X* to cm. 2*R* (as in (*b*) above)
Tip 4-102/86*E*-87*B*/76*A*-80/91*A*-93*F*/40-cm. 2*L*
Tip 2*R*-56*B*/42*C*-49*F*/81-84*F*/98*A*-98*E*/13*E*-11*F*/80-cm. 3*L*
Tip 3*L*-64*C*/76*A*-70*C*/49*F*-51*E*/81-cm. 3*R*
Tip 2*L*-24*D*/3*C*-1*B*/5*D*-11*F*/15*F*-13*E*/41-42*C*/33*E*-24*D*/3*C*-5*D*/85*F*-86*E*/102-cm. 4

On this interpretation the entire complex can be resolved into seven independent exchanges or groups of exchanges: four of them each involving two breakage points, one with four, and two others with ten points each. They are the following: 93*F*/40; 96*B*/96*E* (deficiency); 24*D*/3*C*; 102/86*E*; 15*F*/91*A*/80/11*F*; 98*A*/99*D*/99*F*/85*F*/5*D*/1*B*/41/13*E*/98*E*/84*F*; 87*B*/51*E*/81/49*F*/70*C*/33*E*/42*C*/56*B*/64*C*/76*A*.

Although this represents the simplest pattern of recombination, it does not appear on purely cytological grounds to be the most probable one, for the reason that the assumed sequences 80/91*A*, 80/11*F* and 81/51*E*, 81/49*F* have not been observed, as is usually possible in such cases (for example, the sequences related to the 40 and 41 breaks in the second chromosome). There are also some indications that certain of the ends bordering on the chromocenter, as those of sequences *f* and *g*, are associated with material of

the nucleolus, and this suggests that the nucleolus-organizing region may have been transferred from the *X*-chromosome to the chromocenter of another chromosome. Under such conditions, the number of breaks involved would be increased to at least 34, of which as many as 26 might contribute to the formation of a single rearrangement, the remaining 8 pairing in twos. Whatever the actual pattern of recombination may be, the complexity of the rearrangement in itself merits consideration because of the light it sheds on problems of chromosome breakage and recombination.

Discussion.—The most extensive derangement of Drosophila chromosomes previously observed in this laboratory involved 14 breaks. One reason why complex, multiple-break recombinations are encountered much less frequently than the simpler types is that zygotes receiving such altered chromosomes depend for their survival on the chance union of several broken ends to form viable combinations, while the zygotes receiving acentric and dicentric chromosomes perish in the early divisions to provide the dominant lethals represented by unhatched eggs. Calculations made for the less complex rearrangements[1, 2] indicate that with an increasing number of breaks the percentage of viable combinations decreases. Discovery of the rearrangement reported in the present paper has emphasized the need for determining the probability of such a viable combination. Calculations made to this end[3] indicate that the chances for a balanced, viable reassociation of the segments separated by 32 breaks distributed among all the chromosomes are about one in thirty or forty. This frequency is high enough so that in itself it does not account for the failure to detect such changes in the ordinary quantitative experiment involving cytological inspection of several hundred pairs of glands. The fact that no other alterations of equal or greater complexity have come to light indicates that their appearance does not depend solely on chance recombination of fragments, but probably on factors limiting the production of rearrangements involving as many as 32 breaks. One such limiting factor is the number of regions of potential breakage immediately produced by the radiation; another is the chance that some potential breaks may undergo restitution. These questions have been discussed in a separate paper.[3]

Presumably, the points of potential breakage induced by the radiation that penetrates the nucleus are scattered at random along the chromosomes. Break frequency as revealed in the salivary-gland chromosomes has been found to be essentially at random; the breaks considered as a whole are distributed among the chromosomes in proportion to their lengths.[1, 2] Since these findings are based on the analysis of a multitude of rearrangements, mostly with two breaks, the question remains whether the greater complexity of certain alignments might not be related to the accumulation of breaks within individual chromosomes or chromosome limbs. In the

rearrangement here reported, a large proportion of the breaks was found in the right limb of the third chromosome. Considering only the 27 breaks in the essentially euchromatic regions of the longer chromosomes (excluding the proximal heterochromatic zones), the following distribution was found: 6 in the *X*, 2 in 2*L*, 4 in 2*R*, 3 in 3*L*, 12 in 3*R*. If random distribution of ionization and of regions of potential breakage is assumed, as seems entirely justifiable from available physical evidence, and if recombination were at random, the observed breaks should also be at random. However, the probability of securing the observed distribution lies between 0.02 and 0.05, using the χ^2 test as a measure of significance. On the basis of this low probability, the distribution of breaks in this highly complex case might be referable to some factors interfering with randomness of recombination.

As has been pointed out by Bauer, Demerec and Kaufmann,[1] break distribution among the chromosome limbs does not agree with expectation even for the simpler cases. In the two-break cases, a preponderance of inversions was found as compared with translocations, and this has been attributed, in general, to spatial relationships favoring recombination between adjacent regions. Likewise, among the three-break cases there was an excess over expectation of rearrangements with all the breaks in one limb (3), and a dearth of those with each break in a different limb (1 : 1 : 1). Bauer[2] reports, however, that random distribution obtains in the four-break cases. Several complications preclude the application of reliable statistical tests to the high-break cases; they are few in number, and the proportion of expected viables in each group varies according to the number of chromosome limbs involved. Among slides examined by the writer there are 174 multiple-break cases distributed as follows: 98 with four breaks, 34 with 5 breaks, 22 with 6, 7 with 7, 7 with 8, 4 with 9, and 2 with 12 (breaks in the fourth chromosome are not included). Although this material does not lend itself to critical evaluation for the reasons given above, distribution of breaks in the 5- and 6-break cases does not appear to deviate widely from randomness. Casual inspection of the more complex rearrangements indicates a clumping of breaks; for example, one of the twelve-break cases had 8 breaks in one chromosome limb, and two in each of two others (8 : 2 : 2). The other had a 6 : 4 : 2 distribution. It should also be pointed out that, of the 76 rearrangements with five or more breaks, only two involve all five of the chromosome limbs. The overall impression gained from such a survey, although not conclusive, is that the breakage points detected in individual rearrangements (whether simple or complex) are not distributed at random among the chromosomes. In the light of this conclusion, some further consideration seems warranted concerning the mechanism whereby chromosomal rearrangements are produced.

Summary.—Among the larval progeny of an irradiated *Drosophila melanogaster* male there appeared one female whose salivary-gland chromosomes showed extensive rearrangement that involved at least 32 points of breakage, and probably some others. The complexity is worthy of note, since no rearrangement involving more than 14 breaks had previously been discovered in this laboratory. The breaks in this complex rearrangement are not distributed at random, but are aggregated particularly in the right limb of the third chromosome. Such non-random distribution, coupled with the fact of the occurrence of such a complex rearrangement, prompts further consideration of the factors involved in chromosome recombination.

[1] Bauer, H., Demerec, M., and Kaufmann, B. P., *Genetics*, **23**, 610–630 (1938).

[2] Bauer, H., *Chromosoma*, **1**, 343–390 (1939).

[3] Fano, U., *Proc. Nat. Acad. Sci.*, **29**, 12–18 (1943). (Calculations will be found in the Appendix.)

MECHANISM OF INDUCTION OF GROSS CHROMOSOMAL REARRANGEMENTS IN DROSOPHILA SPERMS

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It has become progressively clear during recent years that the action of ionizing radiations on Drosophila sperm chromosomes develops through two fairly distinct stages, namely, "single-atom effects" and "combination effects."[1] Single-atom effects are individual alterations of the chromosomes, resulting in actual or potential breaks; and each of them arises from the activation (excitation or ionization) of a single atom during the treatment. According to physical information, atomic activations are produced at random and independently of each other throughout the chromosomal material and throughout the duration of the irradiation. The products of single-atom effects remain separate from one another during the spermatozoon stage. Combination effects occur after fertilization, when the previously produced single-atom effects become apparent, so that the broken ends derived at the different points of rupture can be shuffled and rejoined in new combinations.

It is generally recognized that the laws governing the single-atom effects are known in their essentials, although the corresponding mechanism of action is not. The laws governing the combination effects are less well known. The simplest hypothesis is that, following fertilization, broken

chromosome ends may join new partners under conditions of free competition. Experimental evidence does not agree with the implications of this hypothesis;[1, 2, 3] therefore it was found necessary to consider mechanisms of "restricted competition." Restrictions on the freedom of competition were thought to arise from spatial and structural relationships between different breaks, but could not be formulated in simple, unequivocal rules. Analogous factors appear to affect the occurrence of chromosomal rearrangements in Tradescantia microspores, where it also appears that chromosomal mechanical stresses may affect differentially the chance for any individual break to reheal.[4] All earlier discussions, whether based on the theory of free or of restricted competition, seem to have carried in the background one unformulated assumption, namely, that the rejoining of one or more pairs of broken ends during the process of recombination does not affect the fate of other broken ends otherwise than by reducing the number of broken ends available for further recombinations. This would mean that the fate of one broken end does not influence actively the subsequent behavior of other broken ends.

In fact, the exclusion of any such active influence is not required by logical argument nor by experimental evidence. Stresses along chromosomes and interchromosomal pulls due to interlocking, for instance, may provide mechanisms by which the joining of two broken ends may actively influence the recombination of other broken ends. Therefore, the earlier "unformulated assumption" may serve as a tentative hypothesis only so long as it is not disproved by further experimental evidence. It has already been pointed out[3] that the relative frequency of simple and complex rearrangements cannot easily be explained on the basis of existing ideas concerning the combination effects. The recent discovery[5] of a complex rearrangement involving at least 32 breaks prompts us now to re-examine the question.

As far as the production of two-break rearrangements is concerned, the present interpretation seems to be satisfactory. The purpose of this paper is to determine how this interpretation should be extended in order to account for the available evidence on complex rearrangements. Two generally recognized results will be taken as a basis: (*a*) The primary action of x-rays consists of single-atom effects; that is, of individual breaks, produced independently of each other. The average total number of breaks is proportional to the radiation dosage, and the number within each sperm is governed by a Poisson distribution. (*b*) The observed frequency of two-break rearrangements is proportional to the square of the dosage. (The latter result holds when the dosage is sufficiently low; that is, when the frequency of all rearrangements is small as compared to one.) Evidence that this situation occurs in Drosophila has repeatedly been re-

viewed,[1] but the most direct evidence on the laws governing the production of individual breaks has been gathered in grasshopper material.[6]

Simple mathematical considerations, which are omitted for the sake of brevity, show that the following can be inferred from (*a*) and (*b*) within their limits of validity. The chance that any break produced within a sperm will become involved in a two-break rearrangement is proportional to the average number of other breaks present in the same sperm. The establishment of this inference represents a positive achievement, but it does not yet yield a sufficiently complete picture of the process of rearrangement. In fact, this picture might be filled in equally well in either of two alternate ways (as well as intermediate ones), namely: (1) Less than two breaks per sperm are frequently produced, in the usual range of dosage, but it is very likely that a two-break rearrangement will occur whenever two breaks happen to be available in the same sperm. (2) On the average, there are many breaks in every sperm, but each break has only a slight chance of combining with any other single break to give rise to a rearrangement. (In this case it must be assumed that most of the breaks "reheal," because otherwise unhealed breaks would cause dominant lethal effects to a much greater extent than has been observed.[7])

To discuss the relationships between two-break and many-break rearrangements, it is well to consider (1) and (2) separately. On the basis of (1), it is possible to estimate from the observed frequency of two-break rearrangements that the total rate of production of breaks should be of the order of 0.4 per sperm per 1000 r x-rays. It is easily seen that this rate is grossly inadequate not only to account for even the occasional occurrence of a 32-break rearrangement within all the x-rayed material ever observed, but also to account for the observed rate of production of less complex rearrangements (e.g., of the 6-break ones). On the basis of (2), the inference established for two-break rearrangements at low dosage appears to represent a principle of "non-interference" between different breaks: the chance for one break to recombine with any other break is not affected by the presence of still other breaks in the same sperm; hence the total chance of recombination of one break is the sum of the chances of its recombining with each of the other breaks in the sperm. This principle of non-interference, which is founded upon experimental evidence as far as two-break rearrangements are concerned, is clearly related to the generally accepted "unformulated assumption" that was discussed above. That this principle applies to complex rearrangements as well may seem plausible; but it remains only a working hypothesis, to be tested by comparison with experimental evidence.

It is not easy to perform this test, because there is no single way in which to develop a mathematical theory from the working hypothesis and thus to obtain theoretical formulae for comparison with quantitative experimental

results. Several fragmentary considerations have been taken into account, however, that permit us to state with some assurance that the working hypothesis should be rejected. One such consideration runs as follows: In order that very complex rearrangements may occur even occasionally, the rate of occurrence of breaks should be much larger than previously suspected; and conversely, the chance for any pair of breaks in the same sperm to give rise to a two-break rearrangement should be very small, about $^1/_{100}$ or even less. Under these conditions and under the assumption of non-interference between different breaks, complex rearrangements should consist almost exclusively of superimposed two-break rearrangements; while the frequency of more complex "contacts" should be negligible. No such extreme effect has been observed.[8] (It is worth noting, however, that more "2 + 2" rearrangements have actually been found than was to be expected on certain other bases.[8])

To account for the experimental evidence on the frequency and type of complex rearrangements, therefore, it seems necessary to assume the existence of an active influence between different breaks. Although the chance for any one break to become involved in a rearrangement is to begin with simply proportional to the average number of other breaks in the same sperm, this chance should become materially greater when other breaks themselves become involved in a rearrangement. The assumption of combination effects of this sort does not seem unreasonable, in view of the situation prevailing when the sperm opens up after fertilization. The establishment of an illegitimate union between different parts of the chromosomal complex may well perturb the mechanical phenomena that are developing, so as to draw into rearrangement "potential" breaks (i.e., single-atom effects) that would have rehealed otherwise. On this basis the non-random concentration of breaks observed in parts of the chromosomal complex[5] could be easily understood, since mechanical perturbations cannot be expected to affect the whole complex uniformly.

Although our assumption may seem fairly satisfactory, it must be stated that the requirements set by the evidence on complex rearrangements are not quite specific, implying only that chromosomal breaks become more readily available to take part in a rearrangement when the rearrangement itself begins to develop from other breaks. Breaks that become more readily available do not logically need to be drawn from the same source (single-atom effects) as the breaks that started the rearrangement. The alternate hypothesis, that breaks are produced by combination effects in some other way does not seem very reasonable in view of general evidence on the behavior of chromosomes; and it would not even be mentioned here, except that some evidence in its favor, even though weak, does exist. The arguments considered earlier in this paper have referred to evidence on the total number of complex rearrangements observed per two-break rearrange-

ment, that is, on the relative frequency of complex and simple rearrangements in general. Data are also available, however, on the variation of this relative frequency with x-ray dosage throughout the experimental range (that is, between 1000 and 5000 r); this variation is found to be small.[3, 8] This further result cannot be easily explained on the assumption that all breaks originate from single-atom effects. Even though single-atom effects are assumed to be very numerous in each sperm at 1000 r, they should be five times as numerous at 5000 r. The number of breaks constituting raw material on which combination effects can draw during the production of complex rearrangements should thus increase substantially with increasing dosage. This would in turn bring about a corresponding increase in the number of breaks observed per rearrangement, that is, an increase in the "average complexity" of rearrangements. A very small variation in the average complexity is, on the contrary, the result anticipated if it is assumed that the supply of breaks for complex rearrangements does not increase proportionally to the dosage, but much more slowly than that, if at all. This assumption would not necessarily mean that the existence of available breaks is wholly unrelated to the radiation treatment, but that it is related to it through some other mechanism than the single-atom effects.

The fact that the average complexity of x-ray-induced rearrangements depends but little on the dosage might occasion some speculation as to the average complexity of spontaneous rearrangements, inasmuch as these rearrangements might be started by single-atom effects that are analogous to, though much less frequent than, those induced by radiation. Very little can be stated on this subject, by reason of the scarcity of experimental data, except that, in proportion to two-break rearrangements, there are certainly more complex ones in x-ray-treated than in untreated material. For instance, there was found, on the average, in treated material one rearrangement among the types classified as "3-" or "4-" or "2 + 2-" cases per every 2.15 two-break rearrangements. Although this ratio does not vary significantly throughout the dosage range from 1000 to 5000 r,[3, 8] none of the spontaneous rearrangements (numbering 50 to 100) ever detected within all species of Drosophila appears to involve more than two breaks. However, the chance of observing occasional more complex rearrangements in nature might have been adversely affected by selection.

Summary.—Evidence on the occurrence of radiation-induced chromosomal rearrangements indicates that breaks become more readily available to take part in a rearrangement after the rearrangement begins to develop from other breaks. The simplest possible mechanism that can be postulated is that a large number of potential breaks is produced in each sperm by the usual x-ray treatment, and that each break has a chance of rehealing that is originally large but can be substantially lessened by some mechanical

perturbation arising after fertilization when a rearrangement happens to be started by two other breaks. Consideration of further experimental evidence may indicate, however, that some of the breaks involved in complex rearrangements may not have been produced initially by radiation.

Appendix.—To evaluate the chance of survival of organisms carrying complex chromosomal rearrangements, the following mathematical problems have been considered. Let l chromosome limbs be broken at b points: (1) in how many different ways can the resulting broken ends rejoin regardless of whether the final configuration contains polycentric or acentric sections? (2) In how many different ways can the same broken ends rejoin under such conditions that the final configuration contains no polycentric or acentric section? The answer to the first question is known to be:[8]

$$1 \times 3 \times 5 \times \ldots \times (2b-1) = \frac{(2b)!}{b!\, 2^b} \qquad (1)$$

The answer to the second question is:

$$(b-1)!\, 2^{b-l} \qquad (2)$$

Assuming that all recombinations arising from b breaks in l limbs are equally probable, the average chance of survival of such a recombination is given by the ratio of (2) to (1), that is:

$$\frac{lb!\,(b-1)!\, 2^{2b-l}}{(2b)!} = \frac{l}{b2^l} \Big/ \binom{2b}{b} \left(\frac{1}{2}\right)^{2b} \qquad (3)$$

Using Stirling's formula, when $b >> 1$, this formula becomes approximately:

$$\frac{l}{2^l} \sqrt{\frac{\pi}{b}} \qquad (3')$$

For example, when $b = 32$, $l = 6$, the quantity (3′) is 0.03. In other words, about 3 per cent of recombinations involving 32 breaks distributed among all chromosomes of *D. melanogaster* should survive.

This calculation does not apply straightforwardly to the evaluation of the probability of observing a rearrangement such as that described by Kaufmann,[5] for several reasons. First, it is not true that all recombinations arising from b breaks in l limbs are equally probable, because the actual distribution of breaks into "contacts" is known to affect the probability of recombination. One should therefore calculate the numbers of viable and unviable recombinations for the special class of rearrangement considered; for instance, for the "10 + 10 + 4 + 2 + 2 + 2" rearrangements. General formulae for this purpose are not available. Second, the calculation per-

formed above does not exclude the possibility that some broken ends may rejoin in the original order. Finally, recombinations involving acentric sections (deficiencies) may occasionally survive when these sections are sufficiently short; this actually happened in the case described by Kaufmann.[5]

We think, however, that the results obtained from (3) or (3′) would not be greatly affected even if all objections could be taken into account quantitatively, and that therefore these formulae may offer a fair indication of the chance of survival of complex rearrangements.

[1] *Cf.* Muller, H. J., *Cold Spring Harbor Symposia*, **9**, 151–167 (1941).
[2] *Cf.* Kaufmann, B. P., *Ibid.*, **9**, 82–92 (1941).
[3] *Cf.* Fano, U., *Ibid.*, **9**, 113–120 (1941).
[4] Sax, K., *Proc. Nat. Acad. Sci.*, **28**, 229–233 (1942).
[5] Kaufmann, B. P., *Ibid.*, **29**, 8–12(1943).
[6] Carlson, J. G., *Ibid.*, **27**, 42–47 (1941).
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THE EFFECT OF CENTRIFUGING UPON THE PRODUCTION OF X-RAY INDUCED CHROMOSOMAL ABERRATIONS

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Previous investigations[1] have shown that differential sensitivity to x-rays of *Tradescantia* microspores seems to be related to factors which change the spatial and structural relations of the chromosomes and alter their capacity for movement. If such mechanical factors are involved, any treatment which would tend to increase the stress or movement of the chromosomes during or immediately following irradiation should increase the incidence of chromosomal aberrations. This hypothesis has been tested by subjecting *Tradescantia* microspores to centrifugal forces during and following x-ray treatment.

The types of aberrations produced by x-rays and the relation between dosage and aberration frequency have been described in an earlier paper.[2] Irradiation at prophase produces chormatid breaks in which one or both sister chromatids may be affected. The aberrations are of two general types: simple deletions or 1-hit aberrations, and fusion or exchanges be-

tween chromatids of different chromosomes, and since these involve two breaks they are classed as 2-hit aberrations. When the cells were irradiated during the resting stage the aberrations observed at the following metaphase include dicentric and ring chromosomes, minute deletions, and occasional simple terminal deletions. Of these only the rings and dicentrics have been included in the present investigation. Prophase stages are much more sensitive than is the resting stage as measured by the incidence of chromosomal aberrations.

An International clinical centrifuge was used for most of the experiments and was operated at 2080 r. p. m. *Tradescantia* inflorescences were wrapped in moist paper and placed at the bottom of the cups. Irradiation while centrifuging the buds presented some difficulty. The aluminum cover and bronze cups of the centrifuge reduced the radiation intensity to 6 roentgens per minute. The dosage could not be measured directly and had to be estimated by the time of exposure, but the relative doses for the centrifuged and control series were checked by placing the dosimeter on the floor to one side of the centrifuge. Dosimeter readings were obtained for exposures made before centrifuging. The dosimeter, built by Dr. Cloud of M. I. T., was designed so that the dosage in roentgen units was registered in the control room.

When raying and centrifuging simultaneously the centrifuge was run continuously in some experiments and in others it was turned on and off alternately every half minute. During the off periods the revolution was sufficient to keep the cups horizontal, although a difference of one or two centimeters would have had little effect on the dosage, since the distance from the x-ray tube to the centrifuge was about 50 cm. In the control experiments the inflorescences were placed in the cups and rayed without centrifuging, but with the cups at the operating level, or the centrifuge was operated just fast enough to keep the cups horizontal—about 400 r. p. m.

When centrifuging followed irradiation, the inflorescences were rayed at 70 roentgens per minute. Centrifuging was started about five minutes after raying and continued for 30 minutes. Both intermittent and continuous centrifuging were used.

A control experiment showed that centrifuging alone had no effect on the frequency of chromosomal aberrations. Microspores which had been centrifuged for 20 minutes at prophase showed only 0.1 per cent of chromatid aberrations, which is approximately the normal frequency for untreated *Tradescantia* microspores.

The effects of centrifuging *Tradescantia* microspores during or after irradiation are shown in table 1. The data presented in part *A* of the table are combined from eight different experiments, covering a period of several months, and involving the analysis of 63,238 chromosomes. Resting cells

which were irradiated while being centrifuged have twice as many aberrations as those which were irradiated only. Irradiation of resting cells followed by centrifuging was no more effective than irradiation alone. There was no effect of either continuous or alternating centrifuging following irradiation.

The effect of centrifuging at the time of irradiation is shown for cells treated at prophase in part *B* of table 1. These data are derived from five different experiments involving 28,110 chromosomes. There is a very definite and significant increase of chromatid aberrations when the cells are centrifuged during irradiation, but no significant increase when the cells were centrifuged following irradiation.

Chromosomal aberration frequencies are increased both in the resting stage and at prophase when irradiation is accompanied by centrifuging the microspores. Centrifuging following irradiation has little or no effect on the incidence of either chromosome or chromatid aberrations. Centrifuging following irradiation might be expected to increase aberration frequency since fractional dosage experiments and the effect of varying x-ray intensity have shown that the production of aberrations may continue for 20–40 minutes after irradiation. There is some increase in chromatid aberrations resulting from centrifuging after raying, but the differences are not statistically significant. The absence of a significant effect when centrifuging follows irradiation may be due in part to the delay of about five minutes in shifting the material to the centrifuge, and in part to a rapid decline of potential fusions after irradiation. Although potential fusions persist in some cases for 20 to 40 minutes, in some experiments all fusions were completed in less than 20 minutes after the cessation of raying.

It has long been known that various cell constituents can be rearranged by centrifuging. Usually the nuclei occupy centrifugal positions, and occasionally nucleoli can be separated from the nucleus. More recently Kostoff[3] found that the chromosomes of a cell can be shifted by centrifugal forces and in some species the chromosomes may be broken by centrifuging at 2500–3500 r. p. m. Even in the resting stage it is probable that centrifugal forces would cause some stress on the chromosomes, either indirectly by the movement of the denser nucleoli, or directly by differential movement of the chromonemata due to spatial relations. At prophase there should be considerable opportunity for induced chromosome movement since at this stage the chromosomes are partially contracted and do not completely fill the nucleus.

Any stress imposed upon the chromosomes during irradiation would be expected to increase the frequency of chromosomal aberrations. Most of the breaks produced by x-rays undergo restitution and no permanent effect is produced. Only when broken ends of chromosomes get out of alignment do illegitimate fusions occur which result in chromatid and chromo-

some aberrations. Stresses induced in the chromosomes at the time of breakage would separate the broken ends and promote illegitimate associations. Presumably this is the mechanism involved in the centrifuging of cells during irradiation.

Summary—Tradescantia microspores which were centrifuged during irradiation with x-rays were found to have a higher frequency of chromosomal aberrations than microspores which were irradiated only. The effect of centrifuging is attributed to the stresses imposed upon the chromosomes which cause the broken ends to separate and facilitate illegitimate unions of broken chromosomes. Thus chromosome movement appears to be an important secondary factor in the production of chromosomal aberrations by x-rays.

TABLE 1

EFFECT OF CENTRIFUGING X-RAYED MICROSPORES

A. Rayed during resting stage. 63,238 chromosomes

DOSE R. U.	METHOD USED*	PER CENT CHROMOSOME BREAKS CONTROL	CENTRIFUGED	DIFF.
±120 r.	C, D	2.2	4.4	2.2 ± 0.24
320 r.	C, F	15.1	16.8	0.5 ± 0.9
320 r.	A, F	16.5	15.9	

B. Rayed at prophase. 130–160 r. 28,110 chromosomes

METHOD USED*	PER CENT CHROMATID BREAKS 1-HIT CONT.	1-HIT CENT.	2-HIT CONT.	2-HIT CENT.	DIFF. 1-HIT	DIFF. 2-HIT
A + C, D	4.6	7.6	8.9	12.1	3.0 ± 0.4	3.2 ± 0.9
A, F	5.4	6.6	6.7	8.0	1.2 ± 1.0	1.3 ± 1.4

* C—continuous centrifuging. A—alternating centrifuging. D—centrifuged during irradiation. F—centrifuged following irradiation.

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*INCREASE IN MAMMARY CARCINOMA INCIDENCE FOLLOWING INOCULATIONS OF WHOLE BLOOD**

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This is a report of an investigation of the possible rôle of blood in influencing the incidence of mammary carcinoma in mice. It is part of some work started in 1941 to try to open up new leads in and to further the study of a non-mendelian (though transferable from generation to generation maternally) influence affecting the incidence of mammary carcinoma.

In this particular experiment we have been able to conclude observations on the first group of mice. The last animals have just died at 736 days of age. Since the earlier tabulations the results have become increasingly significant. The probability that the results are due to chance alone shows odds of 1 to 10,390, that a random sample would give as great or greater deviation.

The recipient and control mice were of the second inbred generation following the foster nursing of inbred "high tumor" Jackson Laboratory C3H mice on "low tumor" inbred C57 black mice.

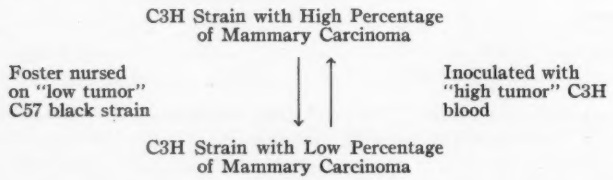
C3H Strain with High Percentage of Mammary Carcinoma
Foster nursed on "low tumor" C57 black strain
Inoculated with "high tumor" C3H blood
C3H Strain with Low Percentage of Mammary Carcinoma

FIGURE 1

Plan of experiment showing method of securing "low tumor" C3H strain and the set-up for attempting to return it to "high tumor" strain.

At weaning time a male and four litter-mate females were placed in each compartment of a mouse box. Breeding was allowed to proceed normally and the young of each litter were removed by the time they were four weeks of age. Two females of each group of four litter-mates were injected subcutaneously with 0.5 cc of whole blood diluted with an equal part of distilled water. The recipients were 1 to 3 months of age. Normal males and females of the Jackson Laboratory C3H high tumor strain were used as donors. Most of the donors were young, non-lactating females, a few were breeding females and the balance were young males. The donors were killed with massive doses of nembutal and the blood secured from the thoracic cavity with a syringe, diluted with warm distilled water and injected

as quickly as possible. The blood of each animal was used individually and not pooled. Following injection into subcutaneous tissue of the back, the blood was spread under the skin with slight pressure. There were 118 mice in the experimental group and 111 in the control group at the time that the first tumor appeared. Animals dying before this age are not included.

Results.—In the control group of 111 females, 11 carcinomas of the mammary glands have appeared at an average age of 351.3 days; 100 mice have died tumor free at an average age of 508.7 days.

In the experimental group of 118 mice, 36 had mammary carcinoma at an average age of 354.0 days; 82 died tumor free at an average age of 460.5 days.

All of the tumors were examined histologically and were found to be adenocarcinomas. There were several mammary gland infections which might grossly have been mistaken for mammary gland tumors. These appeared in both the control and the experimental groups.

There were more than three times as many tumors in the experimental as in the control group. The percentage difference between the two groups was 20.6 with a standard error of the difference of 5.34. An analysis of the difference gives the difference over the standard error of 3.9. The P value was 0.000096. The odds are only 1 to 10,390 that a random sample would give as great or greater deviation. From analysis of the data it was ascertained that there was no litter-mate correlation and thus it was justifiable to use the whole number for comparison.

MICE	NO. OF ANIMALS AT TUMOR AGE	NO. OF TUMORS	% OF TUMORS	AVERAGE AGE AT APPEARANCE OF TUMOR	NO. DYING, TUMOR FREE	AGE DYING, TUMOR FREE
Experimental	118	36	30.51	354.0	82	460.5
Control	111	11	9.91	351.3	100	508.7

$20.60 \pm 5.34 \quad D/\sigma = 3.9 \quad P = 0.000096$

Discussion.—In testing for the significance the question arises whether both groups should be considered from the same date although the first tumor appeared a little later in one group than in the other. The first tumor in the control group appeared at 204 days and in the experimental group at 144 days. The data were examined by starting the control and experimental tabulations at the time of the earliest tumor of either group, that is, at 144 days, and again by starting each tabulation when the first tumor of that group appeared. The results remained significant with either method of calculation.

It is evident that the factor need not come only from breeding or from lactating females, as is shown from the age and sex of the animals used as donors. About four-fifths of the experimental mice received blood from males or immature females. Blood from the immature, non-lactating females, as well as from males, increased the incidence of breast carcinoma.

The blood injection did not return the incidence of mammary carcinoma to the high level of tumor incidence occurring in the Jackson Laboratory C3H strain itself. Instead of the 30% tumor incidence there should have been an incidence of 80% or more had this been so. It might be useful to hypothesize that that age of donor or recipient had something to do with this result. Another possibility is that the amount of blood injected was not sufficient to return the animals to the former high level of incidence. In line with this idea, Bittner, using fostered A strain mice, found that with the feeding of 0.9 cc. of milk 3 out of 10 developed mammary tumors, while with 1.7 cc. of milk, 8 out of 10 developed tumors. If the blood contained the influence in a quantity approximately equal to that in the milk, the 0.5 cc. of whole blood would not be expected to return the tumor incidence to the normal high level. There is some further evidence for interpretation of the data on a quantitative basis. Andervont has shown that high tumor young, suckling their own mothers for 17 hours or less, have a significantly lower mammary tumor incidence following foster nursing by low tumor females than young which have suckled for 24 to 48 hours. Twenty-eight mice suckling less than 17 hours had a tumor incidence of 25% while 23 sucking 24 to 48 hours had an incidence of 69.6%.

Other Tumors.—The second most frequent tumor to appear has been lymphoid leukemia which has now appeared in 25 animals. Fifteen of these were in the control group and at an average age of 585.4 days. Ten were in the treated group at an average age of 543.5 days. The general incidence, 10.1%, is a higher percentage of lymphatic tumors than has usually been recorded for the C3H strain. Appearing at the average age of 568.6 days it is evident that normally many animals which might have produced them were probably eliminated by the earlier appearing mammary gland tumors. Their appearance seems to have no relation to this experimental procedure except the fact that in general the mammary gland tumor incidence was fairly low thus leaving more than a normal proportion of animals to reach the upper age groups.

Summary.—Jackson Laboratory C3H mice which have had their tumor incidence lowered by foster nursing were injected at 1–3 months of age with 0.5 cc. of whole blood from normal high tumor C3H lactating females, young females and males. Significant differences are present between the inoculated mice and their litter-mate controls.

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DETERMINATION OF THE SUBGROUPS OF SMALL INDEX

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It is often less difficult to determine all the subgroups of a given group G which are of a small index, or of a relatively large order, than to determine all those of a small order contained in the group, in view of the fact that the number of the latter is often much larger than the number of the former.[1] In particular, the number of the subgroups of index 2 contained in G is always of the form $2^m - 1$ and each of these subgroups involves all the operators of odd order found in G as well as the commutator subgroup of G. When G is the symmetric group of degree n it is well known that it contains one and only one subgroup of index 2 for every value of n while when G is the alternating group of this degree it never contains any such subgroup. The operators of odd order contained in any group either generate the entire group or they generate an invariant subgroup with respect to which the order of the quotient group is a power of 2.

A group cannot contain as many subgroups of index 2 as it has operators and when it has just one less such subgroup it is of order 2^m, abelian, and of type 1 . All other groups of order g contain less than $g - 1$ subgroups of index 2 and at most $g/2 - 1$ such subgroups. If G contains this latter number of subgroups of index 2 then their cross-cut is of order 2 and G involves operators of order 4 each of whose squares generates this cross-cut. All its cyclic subgroups of order 4 are invariant since they involve the commutator subgroup. If G contains an invariant operator of order 4 then exactly half of its operators are of this order since the products of this operator into its other operators of the same order are either of order 2 or they are the identity, while the products of this operator into the remaining operators of the group are all of order 4. If G contains no invariant operator of order 4 then it contains a subgroup of index 2 composed of its operators which are commutative with one of its operators of this order. Hence there results the following theorem: *If a group of order g contains as many as $g/2 - 1$ subgroups of index 2 its order is of the form 2^m, and if it involves operators of order 4 then at least one-fourth and at most three-fourths of its operators are of this order and all these operators have a common square.*

The number of the subgroups of index s in G is always $g/k - 1$, where k is the order of the cross-cut of all these subgroups. A necessary and sufficient condition that the order of G is a power of 2 is that k is a power of 2. The largest number of subgroups of index 2 in a group whose order is not a power of 2 is therefore $g/3 - 1$. For instance, two of the five groups of order 12 separately contain three subgroups of order 6. One of

these groups is abelian and the other is dihedral. A necessary and sufficient condition that a group contains at least one subgroup of index 2 is that it is not generated by the operators of odd order contained in it since these operators generate an invariant subgroup with respect to which the order of the quotient group is a power of 2, as was noted above.

A subgroup of prime index p contained in G is not necessarily invariant when p is odd and the number of such invariant subgroups in G cannot exceed $(p^\lambda - 1)/(p - 1)$, when p^λ is the highest power of p which divides the order of G. This results directly from the fact that the cross-cut of such invariant subgroups involves the pth power of every operator of G as well as the commutator subgroup of G. If a subgroup of index p is non-invariant under G it belongs to set of p conjugate subgroups which are transformed under G according to a transitive permutation group of degree p and hence G contains an invariant subgroup which corresponds to the identity of this permutation group. In particular, the only symmetric or alternating groups which contain a subgroup of index 3 are those of degrees 3 and 4.

Suppose that the group G contains both an invariant and a non-invariant subgroup of the same prime index p. It was noted above that it will then contain at least one set of p conjugate subgroups which are transformed under it according to a transitive permutation group of degree p with which G is isomorphic. The invariant subgroup of index p which is supposed to be contained in G cannot be simply isomorphic with an invariant proper subgroup of the given transformation group since such a proper subgroup would involve all the permutations of order p contained in this transformation group and hence it could not be of index p under G since the order of this transformation group cannot be divisible by p^2. It therefore results that the given invariant proper subgroup corresponds to all the operators of this transformation group and hence it also contains p conjugate subgroups, p being an odd prime number, and that the order of G is divisible by p^2. Hence there results the following theorem: *If a group contains an invariant subgroup of odd prime index p and also p conjugate subgroups of this index, then the order of the group is divisible by the square of p and this invariant subgroup also contains a set of p conjugate subgroups.*

When G contains a non-invariant subgroup of index p and also an invariant subgroup of a different prime index q, then p must exceed q. In fact, if p were less than q, then G would be isomorphic with a non-regular permutation group of degree p and every invariant subgroup of such a permutation group must involve all its operators of order p and hence it corresponds to a cyclic quotient group whose order divides $p - 1$. The invariant subgroup of index q in G could therefore not correspond to an invariant proper subgroup of this group. It therefore results that p must exceed q and that q must divide $p - 1$. That is, *when a group contains an*

invariant subgroup of prime index and a non-invariant subgroup of a different prime index, then the former of these primes must divide the latter diminished by unity.

If the index of a subgroup of G is composite, its smallest possible value is 4. If G contains invariant subgroups of index 4 the total number of these subgroups has a cross-cut with respect to which the quotient group is abelian and involves no operator whose order exceeds 4. Hence the number of its invariant subgroups of index 4 is equal to the number of subgroups of index 4 in such an abelian group whose order is a power of 2. The given cross-cut involves the commutator subgroup of G since a group of order 4 is necessarily abelian and the order of the quotient group which corresponds to this cross-cut is a divisor of a Sylow subgroup of G whose order is a power of 2. The only symmetric or alternating group which contains a subgroup of index 4 is of degree 4. It is well known that the smallest index of a subgroup of a symmetric or alternating group increases indefinitely as the degree of this group increases indefinitely. The fact that this index could not be less than the degree when this degree exceeds 4 results directly from the fact that the symmetric or the alternating group whose degree exceeds 4 cannot contain an invariant proper subgroup whose index exceeds 2 and this is only possible in the former case.

When G contains a non-invariant subgroup of prime index p it also contains a set of p conjugate subgroups since this subgroup cannot be transformed into itself by any operators of G except those which appear in it but when G contains a non-invariant subgroup of composite index k it is possible for every value of k to construct a group which does not contain k conjugate subgroups of this index. In fact, this is true of every imprimitive group in which the subgroup composed of all the permutations which omit one letter is regular on a number, greater than unity, of letters which divide k. In particular, the octic group contains non-invariant subgroups of index 4 but it does not contain a set of four conjugate subgroups of order 2. It is not possible for a group to have both an invariant subgroup of index 4 and also a complete set of four conjugate subgroups of this index unless the invariant subgroup also contains four conjugate subgroups of index 4 and hence is isomorphic either with the alternating group of degree 4 or with the symmetric group of this degree.

It was observed very early in the development of permutation groups that the symmetic group, as well as the alternating group, of degree 6 contains transitive subgroups of index 6. It may be noted in this connection that whenever a symmetric or an alternating group of degree n contains more than one set of conjugate subgroups of index n then all of these sets except one must be composed of transitive subgroups of degree n. This results directly from the fact that if such subgroups were intransitive then the corresponding symmetric or alternating group would involve

more than one invariant proper subgroup. This is known not to be the case when the degree of the group exceeds 4, and the theorem is known to be true as regards the groups whose degrees are less than 5. Similar considerations as regards the symmetric or the alternating group of a prime degree exhibit the fact that such a group cannot contain more than one set of conjugate subgroups of index n but this fact is included in a more general theorem which will be proved in the following paragraph.

When a transitive unique permutation group of degree n, in which the subgroups composed of all the permutations which omit one letter are of degree n − 1, contains other subgroups of index n which involve no invariant proper subgroup of the entire group then this permutation group admits outer automorphisms and vice versa. This theorem results directly from the fact that this permutation group can be represented as a transitive group of degree n in which such other subgroups are the subgroups composed of all the permutations which omit one letter and hence the group admits an automorphism in which one of these second set of subgroups corresponds to one of the original subgroups which are composed of all the permutations which omit one letter. From this theorem it results directly that no alternating or symmetric group of degree n, when n is not 6, contains more than one set of conjugate subgroups of index n since the group of automorphisms of these groups is known to be the corresponding symmetric group. The connection between automorphisms and the existence of subgroups should be emphasized in this connection. From this theorem it follows directly that the simple group of order 168 admits outer automorphisms, as has also been proved otherwise.

In the *Theory of Group Characters* by D. E. Littlewood, page 147 (1940), it is stated that "the easiest subgroups to find are those with large orders, and these present most difficulty by other methods." The present article implies that this is not always the case.

EXISTENCE OF PERIODIC SOLUTIONS FOR CERTAIN DIFFERENTIAL EQUATIONS

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1. In the present Note we prove the existence of periodic solutions for the equation

$$\frac{d^2x}{dt^2} + g'(x)\frac{dx}{dt} + f(x) = e(t), \tag{1}$$

where $e(t)$ has the period T, and g, f are restricted as stated below. As we shall see, the proof is essentially elementary. The type of equation under discussion generalizes the equation for the response of an electrical series circuit with resistance R, capacity C (both constant) and an inductor with current-flux saturation curve $i = h(\varphi)$. Here (1) is the differential equation of the flux with

$$f = \frac{h}{C}, g(x) = Rh.$$

The function $h(x)$ may be satisfactorily represented by an odd polynomial such that $xh(x) > 0$. This particular case suffices to indicate the importance of the periodic solutions of (1).

THEOREM 1. *The following are sufficient conditions in order that (1) possess a periodic solution of period T:*

I. The derivatives $e'(t)$, $f'(x)$, $g'(x)$ exist for all values of their variables.

II. $e(t)$ has the period T.

III. $\dfrac{f(x)}{x} \to +\infty$ *with* $|x|$.

IV. There exist b, $B > 0$ such that

$$|g(x) - bf(x)| \leq B \cdot |x|.$$

If we set for convenience

$$\frac{f(x)}{x} = F(x), \frac{g(x)}{x} = G(x),$$

then III, IV may be replaced by:

III'. $F(x) \to +\infty$ *with* $|x|$. *(Hence, by IV, $G(x)$ has the same property.)*

IV'. $|G(x) - bF(x)| \leq B$.

2. *Remark 1.* Condition I is merely designed to guarantee the existence and uniqueness of a solution with any given initial values of x and $\frac{dx}{dt}$. It would be possible to replace I by a weaker but less easily described condition.

Remark 2. The restrictions I, ..., IV are manifestly fulfilled by the equation of the series circuit, but not, for instance, by van der Pol's equation, or generally by the relaxation equation[1] with or without periodic disturbance.

Remark 3. Chevalley has proved the existence of periodic solutions for equations (1) with f, g still less restricted than ours. His argument rests likewise upon an analogue of Lemma 1 applied to the phase space (x, v) with ellipses replaced by the curves

$$2u = y^2 + 2k(x),\ k = \int_0^x f(x)dx.$$

However we understand that the proof based on elliptic ovals given below is far simpler than his.

3. *Method.* If we set

$$\frac{dx}{dt} + g(x) = y, \tag{2}$$

then the solution of (1) is equivalent to that of the system consisting of (2) and of

$$\frac{dy}{dt} + f(x) = e(t), \tag{3}$$

and our theorem will follow if we can show that the system possesses a solution (x, y) periodic and of period T.

Consider now any point $P(x, y)$. According to the classical existence theorems there is a unique solution or *trajectory* Γ $(x(t), y(t))$ such that $x(0) = x_0$, $y(0) = y_0$. If Q is the point $(x(T), y(T))$ then $P \to Q$ defines a mapping S of the plane xy into itself and Theorem 1 will follow if we can prove that S has a fixed point. For, owing to the periodicity of $e(t)$, the trajectory Γ is likewise uniquely defined by $x(T) = x_0$, $y(T) = y_0$. Hence if Γ returns to P at time T, it is necessarily periodic.

By Brouwer's fixed point theorem then our theorem will follow from

Lemma 1. There is a closed two-cell mapped into itself by S.

We will prove in fact the more precise

Lemma 2. There is a region bounded by an ellipse which is mapped into itself by S.

4. Consider the definite quadratic form

$$2u = ax^2 - 2xy + by^2,\ ab > 1,\ a > 0. \tag{4}$$

Along a trajectory we have

$$u' = \frac{du}{dt} = (ax - y)(y - xG) + (by - x)(e - xF). \tag{5}$$

Let r, θ be polar coördinates for the plane xy. We first show that Lemma 2 is a consequence of

Lemma 3. When r is above a certain value then $u' < 0$.

In fact assume Lemma 3 to hold for $r > r_0$. Since the distance of the ellipse (4) from the origin $\to +\infty$ with u, we may choose u such that it exceeds r_0, and then Lemma 2 will follow from Lemma 3. Thus everything reduces to proving the latter. The proof will be divided into two parts.

(*a*) We first take an α between 0 and $\frac{\pi}{2}$ and assume $\left|\theta - \frac{\pi}{2}\right|$ or $\left|\theta - \frac{3\pi}{2}\right| \leq \alpha$. If $|x|$ is sufficiently large both G and F are positive, and hence in view of $ab > 1$, for $|x|$ large enough

$$aG - F > \frac{1}{b}(G - bF) > -\frac{B}{b}.$$

Hence there is a positive C such that whatever x we have

$$aG - F > -C$$

and therefore

$$\frac{u'}{r^2} < \{C\cos^2\theta + (a + B)\,|\sin\theta|\,|\cos\theta| - \sin^2\theta\} - \frac{e}{r}(b\sin\theta - \cos\theta).$$

The bracket is a continuous function of θ whose value is -1 for $\theta = \frac{\pi}{2} + k\pi$. Therefore for $|\cos\theta|$ sufficiently small, i.e., choosing α sufficiently small, the bracket will be as near -1 as we please. Since e is bounded we may choose r_1 such that for $r > r_1$ the term $\frac{-e}{r}(b\cos\theta - \sin\theta)$ is arbitrarily small in absolute value. Hence we may choose α, r_1 such that $u' < 0$ under the conditions considered.

(*b*) The point (x, y) is such that $\left|\theta - \frac{\pi}{2}\right|$, and $\left|\theta - \frac{3\pi}{2}\right| > \alpha$, where α is the angle just selected. Evidently

$$\epsilon = \frac{e(b\sin\theta - \cos\theta)}{r\cos^2\theta}$$

is bounded under the conditions here considered. Now

$$\frac{u'}{r^2} = -(aG - F + \epsilon)\cos^2\theta + (a + G - bF)\sin\theta\cos\theta - \sin^2\theta \quad (7)$$

is a quadratic form in $\sin\theta$, $\cos\theta$ whose discriminant

$$4(aG - F + \epsilon) - (a + G - bF)^2 > 4(ab - 1)F + \text{const.} \to +\infty \text{ with } |x|.$$

Therefore the form in (7) is definite negative, and so $u' < 0$ if θ is as stated, and if $r > r_2$, where r_2 is chosen large enough.

We conclude then that Lemma 3 holds for $r > \text{max.}\,(r_1, r_2)$ and so Theorem 1 is proved.

5. *Generalization.* Elliptic or other algebraic ovals may be utilized in many other cases to prove the existence of periodic solutions. Thus consider the system

$$\frac{dx_i}{dt} = X_i(x_1, \ldots, x_n, t) + Y_i(x_1, \ldots, x_n) \quad (8)$$

where X_i, Y_i are polynomials in the x_j with degree $X_i < M =$ degree Y_i and the coefficients of X_i are bounded periodic functions of period T. If there exists a positive definite quadratic form

$$2u = \Sigma a_{ij}x_ix_j$$

such that along a trajectory

$$u' = \Sigma a_{ij}x_iY_j < 0$$

for $r^2 = \Sigma x_i^2$ sufficiently large, then the system (8) possesses a periodic solution.

6. Let us observe finally that instead of $u' < 0$ for r sufficiently large we could merely ask that its sign by constant. For if u increases with t, it decreases with $-t$, and since the transformation $t \to -t$ does not affect periodic solutions, u increasing with t would be acceptable throughout.

[1] See the recent paper by Norman Levinson and O. K. Smith, *Duke Math. Jour.*, 9, 382–403 (1942), where a number of earlier references, notably to van der Pol, von Kármán and Liénard are given.

ON THE EUCLIDEAN CONNECTIONS IN A FINSLER SPACE

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The generalization of the parallelism of Levi-Civita in a Riemann space to a Finsler space has been regarded as one of the most important problems of Finslerian Geometry. For its solution different suggestions were made by J. L. Synge,[1] J. H. Taylor,[2] L. Berwald[3] and E. Cartan.[4] In this note we shall study the problem by employing a different method—the method of equivalence. We shall prove that in a general Finsler space an infinite number of Euclidean connections can be defined, in which the connections defined by other authors are included as particular cases.

Let x^i[5] be the coordinates of an n-dimensional Finsler space, whose fundamental integral is

$$s = \int_{t_0}^{t_1} F\left(x^i, \frac{dx^i}{dt}\right) dt, \tag{1}$$

where F is positively homogeneous of the first order in the last n arguments:

$$F(x^i, \lambda y^i) = \lambda F(x^i, y^i), \; y^i = \frac{dx^i}{dt}, \; \lambda > 0. \tag{2}$$

It is well known that the Pfaffian form

$$\omega = \frac{\partial F}{\partial y^i} dx^i \tag{3}$$

is invariant (under a general point transformation). We adjoin to the coordinates x^i of the space $n(n-1)$ auxiliary variables v_k^α, subjected to the conditions

$$v_k^\alpha y^k = 0, \tag{4}$$

and put

$$v_k^n = \frac{\partial F}{\partial y^k}. \tag{5}$$

Then in the space of all the variables x^i, y^i, v_i^α we have n linearly independent invariant Pfaffian forms, namely,

$$\omega^i = v_k^i dx^k, \tag{6}$$

where $\omega^n = \omega$. It is from the Pfaffian forms ω^i that we shall develop our invariant theory of Finsler spaces, from which the Euclidean connections in the space are derived as consequences.

We introduce the elements u_k^i of the inverse matrix of (v_k^i), so that we have

$$u_j^i v_k^j = v_j^i u_k^j = \delta_k^i. \tag{7}$$

The elements u_n^k do not depend on the auxiliary variables, since a comparison of equations (4), (5), (7) gives

$$u_n^k = \frac{1}{F} y^k. \tag{8}$$

If we form the exterior derivative of ω^n, we see that we can write

$$(\omega^n)' = [\omega^\alpha \omega_\alpha{}^n], \tag{9}$$

where

$$\omega_\alpha{}^n = -u_\alpha^k \frac{\partial^2 F}{\partial y^j \partial y^k} dy^j + \frac{1}{F} u_\alpha^j \left(\frac{\partial F}{\partial x^j} - \frac{\partial^2 F}{\partial x^k \partial y^j} y^k \right) \omega^n + u_\alpha^j u_\beta^k \frac{\partial^2 F}{\partial x^j \partial y^k} \omega^\beta + \lambda_{\alpha\beta}\omega^\beta, \qquad \lambda_{\alpha\beta} = \lambda_{\beta\alpha}, \tag{10}$$

the $n(n-1)/2$ quantities $\lambda_{\alpha\beta}$ being arbitrary.

We shall suppose that the fundamental integral (1) leads to a "regular problem" of the calculus of variations. As is well known, this amounts to assuming that the matrix

$$\left(\frac{\partial^2 F}{\partial y^i \partial y^j} \right)$$

is of rank $n-1$, or, in our notation, that the Pfaffian forms ω^i, ω_α^n are linearly independent.

If we form the exterior derivative of ω^α, we see that the following invariant conditions can be imposed:

$$(\omega^\alpha)' \equiv \delta^{\alpha\beta}[\omega_\beta{}^n \omega^n], \text{ mod. } \omega^\gamma, \tag{11}$$

where $\delta^{\alpha\beta}$ is Kronecker's symbol. The conditions (11) are equivalent to

$$u_\alpha^j u_\beta^k \left(F \frac{\partial^2 F}{\partial y^j \partial y^k} \right) = \delta_{\alpha\beta}, \tag{12}$$

under which the variables u_α^i are not all independent. By carrying out the calculation of $(\omega^\alpha)'$, we get

$$(\omega^\alpha)' - \delta^{\alpha\beta}[\omega_\beta{}^n \omega^n] = [\omega^\beta \omega_\beta{}^\alpha], \tag{13}$$

where

$$\omega_\beta{}^\alpha = v_k^\alpha du_\beta^k - \delta^{\alpha\gamma}\left(u_\gamma^j u_\beta^k \frac{\partial^2 F}{\partial x^j \partial y^k} + \lambda_{\beta\gamma} \right) \omega^n + \mu_{\beta\gamma}^\alpha \omega^\gamma, \tag{14}$$

the quantities $\mu_{\beta\gamma}^{\alpha}$ being symmetric in β, γ and being introduced to get the most general expression for $\omega^{\beta\alpha}$.

Since the variables u_{α}^{i} are connected by the relations (12), the Pfaffian forms ω_{β}^{α} are not linearly independent. In fact, we find that they satisfy the relations

$$\omega_{\alpha\beta} + \omega_{\beta\alpha} \equiv 0, \text{ mod. } \omega^{i}, \omega_{\alpha}^{n},$$

where the quantities $\delta_{\alpha\beta}$ are used to raise and lower indices, thus

$$\omega_{\alpha\beta} = \delta_{\beta\gamma}\omega_{\alpha}^{\gamma}, \ \omega_{\alpha}^{\beta} = \delta_{\alpha}^{\gamma}\omega_{\gamma}^{\beta}, \text{ etc.} \tag{15}$$

Our fundamental result is that we can, by a proper choice of $\lambda_{\alpha\beta}$, $\mu_{\beta\gamma}^{\alpha}$, arrive at the conditions

$$\omega_{\alpha\beta} + \omega_{\beta\alpha} = H_{\alpha\beta}^{\ \gamma}\omega_{\gamma}^{n}, \tag{16}$$

and that under these conditions the quantities $\lambda_{\alpha\beta}$, $\mu_{\beta\gamma}^{\alpha}$, and hence the Pfaffian forms ω_{α}^{n}, ω_{β}^{α} are completely determined. These Pfaffian forms are therefore invariant Pfaffian forms. The quantities $H_{\alpha\beta\gamma}$ constitute the first set of invariants of the Finsler space. Their vanishing signifies that the Finsler space is a Riemann space.

The expressions for the exterior derivatives of ω_{β}^{α}, ω_{α}^{n} will lead to further invariants of the space. To find them we write the equations (9), (13), (16) in the condensed form

$$\left.\begin{aligned} (\omega^{i})' &= [\omega^{j}\omega_{j}^{\ i}], \\ \omega_{ij} + \omega_{ji} &= H_{ijk}\omega^{kn}, \end{aligned}\right\} \tag{17}$$

with the understanding that H_{ijk} is zero when any one of its indices is n. Putting

$$\Omega_{k}^{\ i} = (\omega_{k}^{\ i})' - [\omega_{k}^{\ j}\omega_{j}^{\ i}], \tag{18}$$

we find, by simply applying the "theorem of Poincaré" that the exterior derivative of the exterior derivative of a Pfaffian form is zero, that $\Omega_{k}^{\ i}$ are of the form

$$\Omega_{k}^{\ i} = R_{k\ jl}^{\ i}[\omega^{l}\omega^{j}] + P_{k\ j}^{\ i\ \alpha}[\omega_{\alpha}^{\ n}\omega^{j}], \tag{19}$$

where

$$R_{k\ jl}^{\ i} + R_{k\ lj}^{\ i} = 0 \tag{20}$$

For a function F in our variables its differential dF is a linear combination of ω^{i}, $\omega_{j}^{\ i}$, the coefficients of the linear combination being the "covariant derivatives." The invariants H_{ijk}, $R_{k\ jl}^{\ i}$, $P_{k\ j}^{\ i\ \alpha}$ and their covariant derivatives form a complete system of invariants in the sense that they are sufficient to determine a Finsler space up to a point transformation.

Now we shall enter into the geometrical interpretation of our results. For this purpose we put

$$\pi_{ij} = \omega_{ij} + \gamma_{ij\alpha}\omega^{\alpha n}, \tag{21}$$

and impose the conditions

$$\gamma_{ij\alpha} + \gamma_{ji\alpha} = -H_{ij\alpha}, \tag{22}$$

in order to have

$$\pi_{ij} + \pi_{ji} = 0. \tag{23}$$

We also put

$$\pi^i = \omega^i. \tag{24}$$

The Pfaffian forms π^i, $\pi_j^{\cdot i}$, of which the latter have not yet been completely determined, will then be employed to define the Euclidean connection in the space.

To each set of variables x^i, y^i, v_k^α we attach a Euclidean space of n dimensions with the frame of reference $M\vec{e}_1 \ldots \vec{e}_n$, where M is a point and $\vec{e}_1, \ldots, \vec{e}_n$ are n mutually perpendicular unit vectors through M. The equations

$$\left.\begin{aligned} dM &= \pi^i \vec{e}_i, \\ d\vec{e}_i &= \pi_i^{\cdot j}\vec{e}_j \end{aligned}\right\} \tag{25}$$

then determine the infinitesimal displacement between two neighboring Euclidean spaces or a Euclidean connection. The property of the Euclidean connection depends on the expressions for the following exterior quadratic differential forms

$$\left.\begin{aligned} \Pi^i &= (\pi^i)' - [\pi^j\pi_j^{\cdot i}] \\ \Pi_j^{\cdot i} &= (\pi_j^{\cdot i})' - [\pi_j^{\cdot k}\pi_k^{\cdot i}]. \end{aligned}\right\} \tag{26}$$

And we find

$$\left.\begin{aligned} \Pi^i &= \Omega^i - \gamma_j^{\cdot i\alpha}[\omega^j\omega_\alpha^{\cdot n}], \\ \Pi_j^{\cdot i} &= \Omega_j^{\cdot i} + \gamma_j^{\cdot i\alpha}\Omega_\alpha^{\cdot n} - \gamma_j^{\cdot\alpha\beta}\gamma_\alpha^{\cdot i\rho}[\omega_\beta^{\cdot n}\omega_\rho^{\cdot n}] + [(d\gamma_j^{\cdot i\alpha} + \\ &\qquad \gamma_j^{\cdot i\beta}\omega_\beta^{\cdot\alpha} - \gamma_\beta^{\cdot i\alpha}\omega_j^{\cdot\beta} + \gamma_j^{\cdot\beta\alpha}\omega_\beta^{\cdot i})\omega_\alpha^{\cdot n}]. \end{aligned}\right\} \tag{27}$$

Due to a reason which we shall give later, it is important to impose the condition that Π^i, $\Pi_j^{\cdot i}$ be exterior quadratic differential forms in ω^i, $\omega_\alpha^{\cdot n}$. This gives

$$d\gamma_j^{\cdot i\alpha} + \gamma_j^{\cdot i\beta}\omega_\beta^{\cdot\alpha} - \gamma_\beta^{\cdot i\alpha}\omega_j^{\cdot\beta} + \gamma_j^{\cdot\beta\alpha}\omega_\beta^{\cdot i} \equiv 0, \text{ mod. } \omega^k, \omega_\alpha^{\cdot n}. \tag{28}$$

Summing up, the conditions on $\gamma_{ij\alpha}$ are (22), (28). We shall satisfy (22) by supposing

$$\left.\begin{aligned} \gamma_{in\alpha} &= \gamma_{ni\alpha} = 0, \\ \gamma_{\rho\sigma\alpha} + \gamma_{\sigma\rho\alpha} &= -H_{\rho\sigma\alpha}. \end{aligned}\right\} \tag{29}$$

Then (28) is reduced to the following more symmetrical form:

$$d\gamma_{\rho\sigma\alpha} + \gamma_{\rho\sigma\beta}\omega^{\beta}_{\cdot\alpha} + \gamma_{\beta\sigma\alpha}\omega^{\beta}_{\cdot\rho} + \gamma_{\rho\beta\alpha}\omega^{\beta}_{\cdot\sigma} \equiv 0, \text{ mod. } \omega^{i}, \omega_{\alpha}^{n}. \quad (30)$$

It is important to note that $\gamma_{\rho\sigma\alpha}$ have naturally to be invariants. An example of such a set of invariants is furnished by $H_{\rho\sigma\alpha}$. We can easily verify that the condition (30) for $\gamma_{\rho\sigma\alpha}$ is equivalent to saying that $\gamma_{\rho\sigma\alpha}$ is of the form

$$\gamma_{\rho\sigma\alpha} = G_{ijk}u^{i}_{\rho}u^{j}_{\sigma}u^{k}_{\alpha}, \quad (31)$$

where G_{ijk} are functions of x^{m}, y^{m} only.

To each set of invariants $\gamma_{\rho\sigma\alpha}$ satisfying the conditions (29), (30) we have the set of Pfaffian forms π^{i}, π^{i}_{j}. With these Pfaffian forms the equations (25) define a Euclidean connection, which gives the infinitesimal displacement between the Euclidean spaces attached to two neighboring sets of variables x^{i}, y^{i}, v^{α}_{k}. Owing to the property that Π^{i}, Π^{i}_{j} are exterior quadratic differential forms in ω^{i}, ω_{α}^{n}, it follows that Π^{i}, Π^{i}_{j} are zero, when x^{i}, y^{i} are given as functions of a parameter t:

$$x^{i} = x^{i}(t), \; y^{i} = y^{i}(t). \quad (32)$$

Hence along a one-parameter family of contact elements (x^{i}, y^{i}) the system of equations (25) is completely integrable and the Euclidean spaces attached can be developed in one and the same Euclidean space. This is essentially the generalization of the well-known theorem of Fermi to a Finsler space. From this fact we see that we can regard the Finsler space as formed by the $(2n - 1)$-parameter family of its contact elements and define a Euclidean connection in the space, with a "tangent Euclidean space" attached to each contact element. There are as many Euclidean connections in the space as there are invariants satisfying our conditions. If we take

$$\gamma_{\rho\sigma\alpha} = -{}^{1}/_{2}H_{\rho\sigma\alpha}, \quad (33)$$

we get the Euclidean connection defined by Cartan.

In conclusion, we remark that each of our Euclidean connections has the property that the equivalence of the Euclidean connection is a necessary and sufficient condition for the equivalence of the Finsler spaces (under point transformations).

[1] Synge, J. L., *Trans. Amer. Math. Soc.*, **27**, 61–67 (1925).

[2] Taylor, J. H., *Ibid.*, **27**, 246–264 (1925).

[3] Berwald, L., *Atti Congr. Mat., Bologna*, **4**, 263–270 (1928).

[4] Cartan, E., *Les espaces de Finsler*, Paris, 1934.

[5] It is agreed, throughout this paper, that a Latin index runs from 1 to n and a Greek index from 1 to $n - 1$.

A GENERALIZATION OF THE PROJECTIVE GEOMETRY OF LINEAR SPACES

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The projective geometry in a space of n dimensions may be briefly described as the geometry of the points and the straight lines in the space. For $n > 2$ the straight lines can be replaced by the linear spaces of a fixed dimension r, $1 \leqq r \leqq n-1$. We have, in fact, the theorem that a one-to-one point transformation in the space, which carries the linear spaces of dimension r into themselves, is a projective transformation. The number of parameters on which the linear spaces of dimension r depend is equal to $N = (r+1)(n-r)$. The geometry in a space of n dimensions in which there is given a family of r-dimensional varieties depending on N parameters is therefore in a certain sense a generalization of projective geometry. In this note we shall show that in such a space a projective connection can be defined. The geometry of paths[1] is a particular case of this geometry for $r = 1$, while the case $r = n-1$ has been studied by M. Hachtroudi[2]

Let $x^1, \ldots, x^n$ be the coördinates in the space and let the family of varieties be defined by a completely integrable Pfaffian system of the form[3]

$$\left.\begin{aligned} dx^i - p^i_\alpha dx^\alpha &= 0, \\ dp^i_\alpha - r^i_{\alpha\beta} dx^\beta &= 0, \end{aligned}\right\} \tag{1}$$

where $r^i_{\alpha\beta}$ are functions of x^α, x^i, p^i_α. Before defining the projective connection in question, we shall develop the invariant theory of the family of varieties under non-singular point transformations

$$\left.\begin{aligned} x^{1*} &= x^1(x^1, \ldots, x^n) \\ &\cdots\cdots\cdots\cdots\cdots \\ x^{n*} &= x^n(x^1, \ldots, x^n). \end{aligned}\right\} \tag{2}$$

The left-hand members of (1) are not invariant Pfaffian forms. To derive invariants or invariant Pfaffian forms of the family of varieties the following device will be repeatedly applied: We adjoin to the variables x^α, x^i, p^i_α the new variables u^i_j, u^α_β, u^α_j, $u^{i\beta}_{\alpha j}$, $u^i_{\alpha j}$. Then the Pfaffian forms

$$\begin{aligned} \omega^\alpha &= u^\alpha_\beta dx^\beta + u^\alpha_j (dx^j - p^j_\beta dx^\beta), \\ \omega^i &= u^i_j (dx^j - p^j_\beta dx^\beta), \\ \omega^i_\alpha &= u^{i\beta}_{\alpha j}(dp^j_\beta - r^j_{\beta\gamma} dx^\gamma) + u^i_{\alpha j}(dx^j - p^j_\beta dx^\beta) \end{aligned} \tag{3}$$

are invariant (in the space of all the variables). The number of the new variables can sometimes be reduced by invariant conditions. In fact, we shall suppose

$$(\omega^i)' \equiv [\omega^\alpha \omega^i_\alpha], \text{ mod. } \omega^j, \tag{4}$$

which are equivalent to the conditions

$$u^{i\beta}_{\alpha j} = u^i_j v^\beta_\alpha, \tag{5}$$

v^β_α being defined by the relations

$$u^\gamma_\alpha v^\beta_\gamma = v^\gamma_\alpha u^\beta_\gamma = \delta^\beta_\alpha. \tag{6}$$

Suppose the conditions (4) or (5) be satisfied. Then we can write, by introducing the new Pfaffian forms φ^α_β, φ^α_j, φ^i_j, the exterior derivatives of ω^α, ω^i in the forms

$$\left.\begin{aligned}(\omega^\alpha)' &= [\varphi^\alpha_\beta \omega^\beta] + [\varphi^\alpha_j \omega^j],\\ (\omega^i)' &= [\varphi^i_j \omega^j] + [\omega^\alpha \omega^i_\alpha].\end{aligned}\right\} \tag{7}$$

The fact that the exterior derivatives of $(\omega^\alpha)'$, $(\omega^i)'$ are zero gives

$$\left.\begin{aligned}[(\varphi^i_j{}' - \varphi^i_k \varphi^k_j - \varphi^\alpha_j \omega^i_\alpha)\omega^j] - [(\omega^i_\alpha{}' + \varphi^\beta_\alpha \omega^i_\beta - \varphi^i_j \omega^j_\alpha)\omega^\alpha] &= 0,\\ [(\varphi^\alpha_j{}' - \varphi^\alpha_\beta \varphi^\beta_j - \varphi^\alpha_k \varphi^k_j)\omega^j] + [(\varphi^\alpha_\beta{}' - \varphi^\alpha_\gamma \varphi^\gamma_\beta + \varphi^\alpha_j \omega^j_\beta)\omega^\beta] &= 0.\end{aligned}\right\} \tag{8}$$

The first equation shows that the expression $(\omega^i_\alpha)' + [\varphi^\beta_\alpha \omega^i_\beta] - [\varphi^i_j \omega^j_\alpha]$ contains ω^j or ω^β in each of its terms. Since the system

$$\omega^i = 0, \quad \omega^i_\alpha = 0 \tag{9}$$

is equivalent to (1) and is hence completely integrable, we see that we can put

$$(\omega^i_\alpha)' = -[\varphi^\beta_\alpha \omega^i_\beta] + [\varphi^i_j \omega^j_\alpha] + [\varphi^i_{\alpha j} \omega^j] + Q^{i\beta}_{\alpha\gamma j}[\omega^j_\beta \omega^\gamma], \tag{10}$$

where $\varphi^i_{\alpha j}$ are new Pfaffian forms and where

$$Q^{i\beta}_{\alpha\gamma j} = Q^{i\beta}_{\gamma\alpha j}. \tag{11}$$

Let us see whether $Q^{i\beta}_{\alpha\gamma j}$ are invariants. For this purpose notice that the Pfaffian forms φ^α_β, φ^i_j in (7) are determined up to the transformation

$$\left.\begin{aligned}\varphi^{\alpha *}_\beta &= \varphi^\alpha_\beta + a^\alpha_{\beta\gamma}\omega^\gamma + a^\alpha_{\beta j}\omega^j,\ a^\alpha_{\beta\gamma} = a^\alpha_{\gamma\beta},\\ \varphi^{i *}_j &= \varphi^i_j + a^i_{jk}\omega^k,\ a^i_{jk} = a^i_{kj},\end{aligned}\right\} \tag{12}$$

where the a's are arbitrary. When φ^α_β, φ^i_j are replaced, respectively, by $\varphi^{\alpha *}_\beta$, $\varphi^{i *}_j$, the equation (10) retains its form, while $Q^{i\beta}_{\alpha\gamma j}$ are replaced by $Q^{i\beta *}_{\alpha\gamma j}$, related to $Q^{i\beta}_{\alpha\gamma j}$ as follows:

$$Q^{i\beta *}_{\alpha\gamma j} = Q^{i\beta}_{\alpha\gamma j} - \delta^i_k a^\beta_{\alpha\gamma}.$$

This shows that $Q^{i\beta}_{\alpha\gamma j}$ are not invariants. We can, however, modify them such that the conditions

$$Q^{i\beta}_{\alpha\gamma j} = 0 \tag{13}$$

are satisfied. Under these conditions $Q^{i\beta}_{\alpha\gamma j}$ are invariants.

If we carry out the calculation of the expression for $Q^{i\beta}_{\alpha\beta j}$, we see that, by a proper choice of $u^i_{\alpha j}$, the conditions

$$Q^{i\beta}_{\alpha\beta j} = 0 \tag{14}$$

can be fulfilled. This determines $u^i_{\alpha j}$ in terms of x^α, x^i, p^i_α, u^i_j, u^α_β, and parameters or new variables which we denote by v_α. When $r = 1$ or $r = n - 1$, the conditions (13) and (14) signify that all $Q^{i\beta}_{\alpha\gamma j}$ vanish.

The conditions (13) and (14) have an effect on the expressions for $(\varphi^\alpha_\beta)'$, $(\omega^i_\alpha)'$. To find these expressions we apply exterior differentiation to (10), which gives

$$[\{\delta^i_j(-\varphi^{\beta\prime}_\alpha - \varphi^\gamma_\alpha\varphi^\beta_\gamma) + \delta^\beta_\alpha(\varphi^{i\prime}_j - \varphi^i_k\varphi^k_j) - \pi^{i\beta}_{\alpha\gamma j}\omega^\gamma - \varphi^i_{\alpha j}\omega^\beta + Q^{i\rho}_{\alpha\gamma k}Q^{k\beta}_{\rho\sigma j}\omega^\sigma\omega^\gamma\}\omega^j_\beta] \equiv 0, \text{ mod. } \omega^k,$$

where

$$\pi^{i\beta}_{\alpha\gamma j} = dQ^{i\beta}_{\alpha\gamma j} - Q^{k\beta}_{\alpha\gamma j}\varphi^i_k + Q^{i\beta}_{\alpha\gamma k}\varphi^k_j - Q^{i\rho}_{\alpha\gamma j}\varphi^\beta_\rho + Q^{i\beta}_{\rho\gamma j}\varphi^\rho_\alpha + Q^{i\beta}_{\alpha\rho j}\varphi^\rho_\gamma.$$

From (8) we get respectively

$$(\varphi^i_j)' - [\varphi^i_k\varphi^k_j] \equiv [\varphi^\alpha_j\omega^i_\alpha] - [\varphi^i_{\alpha j}\omega^\alpha], \text{ mod. } \omega^k,$$
$$(\varphi^\alpha_\beta)' - [\varphi^\alpha_\gamma\varphi^\gamma_\beta] + [\varphi^\alpha_j\omega^j_\beta] \equiv [\theta^\alpha_{\beta\gamma}\omega^\gamma], \text{ mod. } \omega^k,$$

where $\theta^\alpha_{\beta\gamma}$ are newly introduced Pfaffian forms. Substituting these expressions into the last equation, we shall get

$$\delta^i_j\theta^\beta_{\alpha\gamma} + \delta^\beta_\alpha\varphi^i_{\gamma j} + \delta^\beta_\gamma\varphi^i_{\alpha j} + \pi^{i\beta}_{\alpha\gamma j} \equiv 0, \quad \text{mod. } \omega^\alpha, \omega^i, \omega^i_\alpha.$$

The conditions (13) and (14) have as consequences

$$\pi^{j\beta}_{\alpha\gamma j} = 0, \quad \pi^{i\beta}_{\alpha\beta j} = 0,$$

so that the above equation gives, respectively,

$$(n - r)\theta^\beta_{\alpha\gamma} + \delta^\beta_\alpha\varphi_\gamma + \delta^\beta_\gamma\varphi_\alpha \equiv 0, \quad \text{mod. } \omega^\alpha, \omega^i, \omega^i_\alpha,$$

$$\varphi^i_{\alpha j} \equiv \frac{1}{n - r}\delta^i_j\varphi_\alpha, \quad \text{mod. } \omega^\alpha, \omega^i, \omega^i_\alpha,$$

where φ_α is an abbreviation of $\varphi^j_{\alpha j}$. It follows that the exterior derivatives of ω^i_α, φ^α_β are of the forms

$$(\omega^i_\alpha)' = -[\varphi^\beta_\alpha\omega^i_\beta] + [\varphi^i_j\omega^j_\alpha] + \frac{1}{n - r}[\varphi_\alpha\omega^i] + \Omega^i_\alpha, \tag{15}$$

$$(\varphi_\beta^\alpha)' \equiv [\varphi_\gamma^\alpha \varphi_\beta^\gamma] - [\varphi_j^\alpha \omega_\beta^j] - \frac{1}{n-r}\delta_\beta^\alpha[\varphi_\gamma\omega^\gamma] - \frac{1}{n-r}[\varphi_\beta\omega^\alpha] + R_{\beta\gamma\rho}^\alpha[\omega^\rho\omega^\gamma] + R_{\beta\gamma j}^{\alpha\rho}[\omega_\rho^j\omega^\gamma], \text{ mod. } \omega^i, \tag{16}$$

where

$$\Omega_\alpha^i = Q_{\alpha j\beta}^i[\omega^\beta\omega^j] + Q_{\alpha jk}^i[\omega^k\omega^j] + Q_{\alpha jk}^{i\beta}[\omega_\beta^k\omega^j] + Q_{\alpha\gamma j}^{i\beta}[\omega_\beta^j\omega^\gamma]. \tag{17}$$

It is to be noticed that the Pfaffian forms φ_β^α, φ_j^i, φ_j^α, φ_α introduced above are not invariant, because they are not completely determined by the equations (7), (15). These Pfaffian forms are determined up to a transformation which can easily be written down. By investigating the effect of this transformation on the coefficients in (15), (16), it is not difficult to show that we can choose the φ's such that we have

$$Q_{\alpha j\beta}^j = 0, \quad Q_{\alpha jk}^{j\beta} = 0, \quad R_{\beta\gamma j}^{\alpha\gamma} = 0, \quad Q_{\beta jk}^{i\beta} = 0. \tag{18}$$

Under these conditions the Pfaffian forms in question are determined up to the transformation

$$\left.\begin{aligned}
\varphi_\beta^{\alpha*} &= \varphi_\beta^\alpha + \frac{1}{n-r+1}\delta_\beta^\alpha a_j\omega^j,\\
\varphi_j^{\alpha*} &= \varphi_j^\alpha + \frac{1}{n-r+1}a_j\omega^\alpha + a_{jk}^\alpha\omega^k,\ a_{jk}^\alpha = a_{kj}^\alpha,\\
\varphi_j^{i*} &= \varphi_j^i + \frac{1}{n-r+1}(\delta_j^i a_k\omega^k + a_j\omega^i),\\
\varphi_\alpha^* &= \varphi_\alpha + a_{\alpha j}\omega^j + \frac{n-r}{n-r+1}a_j\omega_\alpha^j,
\end{aligned}\right\} \tag{19}$$

where the a's are arbitrary.

We now take v_α, a_j to be new variables and adjoin them to the set of variables x^α, x^i, p_α^i, u_j^i, u_β^α, u_j^α. All these $n(n+2)$ variables then form the set which we shall deal with and by invariance we shall mean the invariance under a general transformation in these variables. It is in this sense that the Pfaffian forms φ_β^α, φ_j^i are invariant. In order to find a set of linearly independent invariant Pfaffian forms, whose number is equal to the number of variables, we form the exterior derivatives of φ_β^α, φ_j^i. From (8), (10) we see that we can set

$$\left.\begin{aligned}
(\varphi_j^i)' - [\varphi_k^i\varphi_j^k] - [\varphi_j^\alpha\omega_\alpha^i] + \frac{1}{n-r}\delta_j^i[\varphi_\alpha\omega^\alpha] - Q_{\alpha j\beta}^i[\omega^\alpha\omega^\beta] - Q_{\alpha jk}^i[\omega^\alpha\omega^k] - Q_{\alpha jk}^{i\beta}[\omega^\alpha\omega_\beta^k] &= [\theta_{jk}^i\omega^k],\\
(\varphi_\beta^\alpha)' - [\varphi_\gamma^\alpha\varphi_\beta^\gamma] + [\varphi_j^\alpha\omega_\beta^j] + \frac{1}{n-r}\delta_\beta^\alpha[\varphi_\gamma\omega^\gamma] + \frac{1}{n-r}[\varphi_\beta\omega^\alpha] - R_{\beta\gamma\rho}^\alpha[\omega^\rho\omega^\gamma] - R_{\beta\gamma j}^{\alpha\rho}[\omega_\rho^j\omega^\gamma] &= [\theta_{\beta j}^\alpha\omega^j],
\end{aligned}\right\} \tag{20}$$

where θ^i_{jk}, $\theta^\alpha_{\beta j}$ are newly introduced Pfaffian forms, of which the former ones are subjected to the conditions

$$[\theta^i_{jk}\omega^j\omega^k] = 0. \tag{21}$$

We form the equation obtained by exterior differentiation of (15)'. A complete analysis of that equation will give the following two consequences:

$$\delta^i_k\theta^\beta_{\beta j} - r\theta^i_{kj} - \frac{1}{n-r}\delta^i_j(\theta^\beta_{\beta k} - r\theta_k) \equiv 0, \quad \text{mod. } \omega^\alpha, \omega^i, \omega^i_\alpha; \tag{22}$$

$$\varphi_\alpha{}' \equiv -[\varphi^\beta_\alpha\varphi_\beta] - [\theta^\beta_{\alpha k}\omega^k_\beta] + [\theta_k\omega^k_\alpha] - Q^{j\beta}_{\alpha\gamma k}[\varphi^\gamma_j\omega^k_\beta] + U^{\beta}_{\alpha\gamma k}[\omega^k_\beta\omega^\gamma], \text{mod. } \omega^i + U_{\alpha\beta\gamma}[\omega^\beta\omega^\gamma] \tag{23}$$

where $\theta_k = \theta^j_{kj}$. On the other hand, by exterior differentiation of the second equation of (20), we can get

$$Q^{k\alpha}_{\beta\sigma j}\varphi^\sigma_k + \delta^\alpha_\beta\theta^\gamma_{\gamma j} - (r+1)\delta^\alpha_\beta\theta_j + (n+1)\theta^\alpha_{\beta j} \equiv 0, \text{ mod. } \omega^\beta, \omega^i, \omega^i_\alpha. \tag{24}$$

From (22) and (24) we then find

$$\theta^\alpha_{\beta j} \equiv \frac{1}{n-r+1}\delta^\alpha_\beta\theta_j - \frac{1}{n+1}Q^{k\alpha}_{\beta\sigma j}\varphi^\sigma_k, \text{ mod. } \omega^\alpha, \omega^i, \omega^i_\alpha, \tag{25}$$

$$\theta^i_{kj} \equiv \frac{1}{n-r+1}(\delta^i_j\theta_k + \delta^i_k\theta_j), \text{ mod. } \omega^\alpha, \omega^i, \omega^i_\alpha. \tag{26}$$

In this way we have introduced the Pfaffian forms θ_k, which involve da_k and are linearly independent from ω^α, ω^i, ω^i_α, φ^α_β, φ^i_j, φ^α_j, φ_α. The total number of linearly independent Pfaffian forms is now equal to the number of variables. But of these forms φ^α_j, φ_α, θ_k are not yet invariant. To derive invariant Pfaffian forms from them, further conditions are necessary. We put, according to (25), (26),

$$\left.\begin{aligned}
[\theta^i_{jk}\omega^k] &= \frac{1}{n-r+1}(\delta^i_j[\theta_k\omega^k] + [\theta_j\omega^i]) + S^i_{jkl}[\omega^l\omega^k] + S^i_{jk\gamma}[\omega^\gamma\omega^k] + S^{i\gamma}_{jkl}[\omega^l_\gamma\omega^k],\\
[\theta^\alpha_{\beta j}\omega^j] &= -\frac{1}{n+1}Q^{k\alpha}_{\beta\sigma j}[\varphi^\sigma_k\omega^j] + \frac{1}{n-r+1}\delta^\alpha_\beta[\theta_j\omega^j] + R^\alpha_{\beta j\gamma}[\omega^\gamma\omega^j] + R^\alpha_{\beta jk}[\omega^k\omega^j] + R^{\alpha\gamma}_{\beta jk}[\omega^k_\gamma\omega^j].
\end{aligned}\right\} \tag{27}$$

It is then easy to show that under the conditions

$$S^j_{kj\gamma} = 0,\ S^{j\gamma}_{kjl} = 0,\ R^\beta_{\beta j\gamma} = 0,\ R^{\beta\alpha}_{\beta jk} + R^{\beta\alpha}_{\beta kj} = 0 \tag{28}$$

the Pfaffian forms φ^α_j, φ_α are completely determined. The uniqueness of θ_k depends on the expression for $(\varphi^\alpha_j)'$. We find

$$(\varphi_j^\alpha)' = [\varphi_k^\alpha \varphi_j^k] + [\varphi_\beta^\alpha \varphi_j^\beta] + \frac{1}{n-r+1}[\theta_j \omega^\alpha] - \frac{1}{n+1} Q_{\beta\sigma j}^{k\alpha}[\varphi_k^\sigma \omega^\beta] -$$

$$\frac{1}{2}(Q_{\beta jk}^{i\alpha} + Q_{\beta kj}^{i\alpha})[\varphi_i^\beta \omega^k] - \frac{n-r+1}{2(n+1)}(R_{\beta\rho k}^{\beta\alpha}[\varphi_j^\rho \omega^k] + R_{\beta\rho j}^{\beta\alpha}[\varphi_k^\rho \omega^k]) +$$

$$R_{\beta j\gamma}^{\alpha}[\omega^\gamma \omega^\beta] + R_{\beta jk}^{\alpha\gamma}[\omega_\gamma^k \omega^\beta] + T_{jk\beta}^{\alpha}[\omega^\beta \omega^k] + T_{jki}^{\alpha}[\omega^i \omega^k] + T_{jki}^{\alpha\beta}[\omega_\beta^i \omega^k]. \quad (29)$$

The conditions

$$T_{jk\beta}^{\beta} = 0 \quad (30)$$

determine θ_k completely.

From the complete determination of φ_j^α, φ_α, θ_k follows their invariance. We have therefore a set of linearly independent invariant Pfaffian forms whose number is equal to the number of variables. A necessary and sufficient condition for two families of varieties to be equivalent is that a transformation in all our variables exists, which carries one set of Pfaffian forms to the other. Hence the problem of equivalence is solved.

If the given family of varieties is the family of linear spaces, our Pfaffian forms are those which define the infinitesimal transformation between two neighboring projective frames. In the general case they define a projective connection. The actual calculation of the components of the projective connection in terms of the coördinates of the space offers no essential difficulty.

[1] Eisenhart, L. P., *Non-Riemannian Geometry*, New York, 1927.

[2] Hachtroudi, M., *Les espaces d'éléments à connexion projective normale*, Paris, 1937.

[3] We agree that Greek indices run from 1 to r and that Latin indices run from $r+1$ to n.

THE EPIDEMIC CURVE, II

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In a previous note[1] we discussed comparatively the formulations by Soper and by Frost of the progress of an epidemic in the absence of recruitment of susceptibles and came to a relationship between the initial susceptibles S_0, the residual susceptibles S_E and the number m which on Soper's formulation was the number of susceptibles for a steady state (when there was recruitment). To discuss periodicity Soper made certain approximations which carried his equations over from algebraic form to expressions in terms of the calculus (differential equations). He introduced a variable $u = \log(z/A)$, where z is rate of cases and A is rate of recruits.

This transformation cannot be used if $A = 0$. We wish now to compare the results of Soper's approximation for the case where $A = 0$ with the results we obtained previously.

If τ be the incubation period and $u = \log z$, Soper's differential equations become

$$1 + \tau \frac{du}{dt} = \frac{S}{m} \text{ and } S = m \text{ for } u = \text{maximum.}$$

Then

$$\frac{dS}{dt} = A - z, \text{ and } \frac{d^2u}{dt^2} = \frac{A - e^u}{m}. \tag{1}$$

The first integral is

$$\tau \left(\frac{du}{dt}\right)^2 = \frac{2}{m}(Au - e^u) + \text{const.}$$

and if the constant is determined when $du/dt = 0$ and $u = u_0$, $t = 0$,

$$t = -\sqrt{\frac{m\tau}{2}} \int_{u_0}^{u} \frac{du}{\sqrt{A(u - u_0) - (e^u - e^{u_0})}}$$

$$S - m = \int_0^t (A - z)dt = At \pm \sqrt{\frac{m\tau}{2}} \int_{u_0}^{u} \frac{du}{\sqrt{A(u - u_0) - (e^u - e^{u_0})}},$$

the integral having the negative sign so that $S < m$ when $t > 0$ and the positive sign with $S > m$ when $t < 0$.

Now if $A = 0$, the integrations may be performed in finite form and in terms of z become

$$z = z_m \operatorname{sech}^2 \sqrt{\frac{z_m}{2m\tau}}\, t$$

$$S = m - \sqrt{2mz_m\tau} \tanh \sqrt{\frac{z_m}{2m\tau}}\, t.$$

The epidemic curve begins at $t = -\infty$ and runs to $t = +\infty$ instead of being of finite duration, but actually since the number of cases cannot be fractional the curve would be limited for practical purposes to a finite interval of time. The curve of the case rate is symmetrical with respect to its maximum whereas the curves figured stepwise from generation to generation were found to be asymmetrical with the decreasing more abrupt than the increasing portion. This is one difference due to the approximations introduced. Another difference is found in the relation between S_0, S_E and m; for, setting $t = \mp\infty$,

$$S_0 = m + \sqrt{2mz_m\tau},\ S_E = m - \sqrt{2mz_m\tau}$$

and

$$S_0 + S_E = 2m \text{ instead of } \frac{S_E}{S_0} = e^{-\frac{S_0}{m}\left(1 - \frac{S_E}{S_0}\right)}.$$

From the second equation we found in tabular form relationships between S_0/m and S_E/S_0 with no restriction on S_0/m. (This was for Frost's formulation as modified by Wilson through the introduction of the law of small numbers; it was impossible to obtain such a relation for Soper's original finite stepwise formulation.) On Soper's formulation in differential equations, we see that since $S_E \geqq 0$, $S_0 \leqq 2m$ and further that $m \geqq 2z_m\tau$.

When therefore we have $A > 0$, as is necessary for periodicity, we cannot expect the stepwise calculations to give the same result as the theory based on Soper's differential equations. For discussing the periodicity ($A > 0$) we will do well to return to Soper's notation, $u = \log(z/A)$. Then

$$t = -\sqrt{\frac{m\tau}{2A}} \int_{u_0}^{u} \frac{du}{\sqrt{u - u_0 - e^u + e^{u_0}}}.$$

The expression $u - u_0 - e^u + e^{u_0}$ has one root u_0 and another root u_1 (at minimum) which may be written approximately[2] as

$$-u_1 = C - e^{-(C - e^{-C})},\quad C = e^{u_0} - u_0.$$

Thus the period is

$$T = \sqrt{\frac{2m\tau}{A}} \int_{u_1}^{u_0} \frac{du}{\sqrt{u - u_0 - e^u + e^{u_0}}}.$$

As the integrand becomes infinite for $u = u_0$ and $u = u_1$ we form the function

$$f(u) = \frac{1}{\sqrt{u - u_0 - e^u + e^{u_0}}} - \frac{1}{\sqrt{(u_0 - u)(e^{u_0} - 1)}} - \frac{1}{\sqrt{(u - u_1)(1 - e^{u_1})}}$$

which remains finite and may be integrated by Simpson's or similar rules, giving[3]

$$T = \sqrt{\frac{2m\tau}{A}} \left[2\sqrt{u_0 - u_1} \left(\frac{1}{\sqrt{1 - e^{u_1}}} + \frac{1}{\sqrt{e^{u_0} - 1}} \right) + \int_{u_1}^{u_0} f(u)du \right].$$

Soper considers a normal intensity for an epidemic to be where $e^{u_0} = 4$ or $z_m/A = 4$, so that the case rate at maximum is four times the rate of recruitment. In many of our epidemics of measles the ratio z_m/A seems larger than 4. For example in Hedrich's epidemic[4] of 1930–1931 in

Baltimore z_m/A as estimated (allowance being made for the incompleteness of reporting) was about 10. In Providence[5] for the 1934–1935 epidemic the maximum number of reported cases in one month was 1953 whereas the recruits were about 350 which gives a ratio of 5.6 but there probably was only about 50% reporting making the true ratio about 11. In Providence in January, 1932, there were 2799 cases reported which gives a ratio of 8.0 and if this be doubled we find 16. A computation of the periods for $e^{u_0} = 4, 6, 10$ shows 7.15, 8.00, 9.75 times $\sqrt{m\tau/A}$. If we take $m\tau/A = 130$ which makes m equal to 5 years of births we find that the periods in the three respective cases would be 3.1, 3.5, 4.3 years.

We can make a stepwise calculation from Soper's finite formulas for the case $S_0 = 36{,}000$, $A = 500$ per incubation period and $m = 29{,}000$; the results are:

TIME	CASES	TIME	CASES	TIME	CASES	TIME	CASES
1	500	15	1106	29	62	43	58
2	621	16	882	30	56	44	65
3	768	17	692	31	51	45	74
4	943	18	538	32	47	46	85
5	1144	19	418	33	44	47	99
6	1362	20	326	34	42	48	116
7	1581	21	256	35	41	49	138
8	1776	22	203	36	41	50	166
9	1917	23	163	37	41	51	201
10	1976	24	133	38	42	52	246
11	1936	25	110	39	44	53	303
12	1801	26	93	40	46	54	376
13	1595	27	80	41	49	55	468
14	1352	28	70	42	53		

In this case $e^{u_0} = 3.95$ and $m\tau/A = 58$ so that the computed period would be $7.15\sqrt{58} = 54.5$ incubation periods or a little over two years. However this makes the value of m only a trifle over two years' births.

There is, however, another point in the theory which is worth mentioning. The maximum u_0 less the minimum u_1 is $u_0 - u_1$ and this is the natural logarithm of the ratio of cases at maximum to cases at minimum which for $e^{u_0} = 4, 6, 10$, respectively, gives 3.92, 5.98, 10.00 of which the antilogarithms are 50, 395, 22,000. The value 50 checks tolerably well with the arithmetical calculation just given. Such a value as 22,000 would of course be impossible except in a very large area because the cases at maximum would have to be 22,000 per incubation period in order for 1 to remain at minimum. In fact the difficulty we have in applying Soper's or Frost's methods to many instances which we actually find in pronounced epidemics is that the cases become fractional and remain so for a long time around minimum—which means that the epidemic would in fact terminate.[6] To be sure of having one case left in Providence we could not have e^{u_0}

greater than 6, nor greater than 7 in Baltimore; whereas when allowance is made for incomplete reporting the values of e^{u_0} are generally greater than those values.

[1] These PROCEEDINGS, **28,** 361–367 (1942).

[2] The equation $u - u_0 - e^u + e^{u_0} = 0$ may be solved numerically for u_1 but the approximation given is generally sufficiently good.

[3] For the case $e^{u_0} = 4$, $u_0 = 1.386$, $u_1 = -2.535$, the total interval is $u_0 - u_1 = 3.921$ and the values of $f(u)$ at intervals of 0.03921 from u_0 to u_1, inclusive, are -0.53, -0.43, -0.39, -0.37, -0.35, -0.33, -0.32, -0.31, -0.30, -0.29, -0.29. The integral of $f(u)$ by Simpson's Rule is -1.37 and the value of T is 7.15 times $\sqrt{m\tau/A}$.

[4] *Amer. Jour. Hygiene,* **17,** 613–636 (1933).

[5] See the Report of the Superintendent of Health of the City of Providence for the year 1940. For convenience the table is reproduced below:

MEASLES CASES BY MONTHS IN PROVIDENCE 1917–1940

YEAR	JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	TOTAL
1917	33	47	62	109	119	36	13	7	2	1	8	55	492
1918	55	98	373	1232	1299	780	261	23	8	6	5	3	4143
1919	1	4	4	4	5	4	3	3	1	2	1	3	35
1920	125	127	136	279	404	288	146	38	45	53	190	191	2022
1921	329	585	665	390	266	99	28	10	1	2	7	26	2408
1922	89	4	3	26	25	22	23	19	7	16	131	652	1017
1923	680	1228	1470	687	383	117	29	6	3	10	7	7	4627
1924	5	6	3	11	16	30	15	2	2	1	5	2	98
1925	13	11	6	15	18	30	58	50	13	81	417	1224	1936
1926	2057	1360	648	348	196	105	48	8	1	0	0	4	4775
1927	5	2	1	1	2	2	6	2	0	9	7	23	60
1928	45	112	422	1081	883	800	508	77	18	36	36	61	4079
1929	84	189	261	399	276	111	38	4	3	2	0	0	1367
1930	2	0	1	4	23	46	22	8	1	0	2	0	109
1931	1	2	49	158	456	358	179	99	22	191	337	1548	3400
1932	2799	2037	574	199	81	11	2	0	0	0	0	0	5703
1933	0	0	0	3	3	6	5	2	4	0	1	1	25
1934	4	11	21	18	29	106	44	25	8	5	1	7	279
1935	13	57	343	1351	1953	1279	241	17	4	1	0	48	5307
1936	119	74	92	76	83	17	11	4	0	0	9	77	562
1937	422	811	1184	711	472	129	31	4	0	2	3	3	3772
1938	2	5	4	2	0	0	0	3	1	0	0	3	20
1939	33	35	40	118	317	286	157	64	20	89	267	446	1872
1940	569	495	530	462	543	372	121	20	1	0	1	1	
Total	7485	7300	6890	7684	7852	4934	1989	495	165	507	1435	4385	51221

Epidemics culminate in May, 1918, March, 1921, March, 1923, January, 1926, April, 1928, January, 1932, May, 1935, March, 1937, March (?), 1940. In this period of 262 months there are 9 major peaks, but we must not count both ends. The average time between peaks is 33 ± 7.9 months, not 2 years. For the mean we write 33 ± 2.8 months. In Glasgow we estimate 40 months between peaks from 1888 to 1927, inclusive, based on Soper's data (*J. Roy. Statist. Soc. London,* **92,** 34–61 (1929)). How many peaks one counts depends on the interpretation one gives to the qualifying adjective major and what allowance one makes for seasonal interruption of an epidemic.

[6] In that case measles would recur only when reintroduced after a lapse of time sufficient to build up a sufficient number of susceptibles, the notion of periodicity based on the continuous operation implied in the differential equations or the stepwise calculation would have to be abandoned, and we should properly speak of recurrences rather than of periods.

The difficulty of discussing the ratio of cases at maximum and minimum, and the matter of continuity versus dying out and reintroduction of measles on the basis of reported cases is great. A fairly good estimate of the fraction of total cases which are reported is easy to make, and if it can be assumed that this ratio is more or less constant the arguments here advanced are sound. There are unpublished data for Newton, Mass., collected by Dr. Harold D. Chope for his doctoral thesis (which is partly published in *Virus and Rickettsial Diseases*, Harvard University Press, 1940, pp. 283–308, under the title "A Study of Factors That Influence Reporting of Measles") which throw some doubt on the propriety of assuming a constant ratio.

MEASLES CASES REPORTED AND NOT REPORTED FOR 1936 AND 1934 BY MONTHS

	JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.–DEC.	TOTAL
Rep.	18	59	204	295	181	75	4	3	839
Not	14	28	61	66	53	38	36	24	320
%	56	68	67	82	77	66	10	11	72
Rep.	25	59	49	69	55	63	17	4	341
Not	25	36	54	56	31	20	37	40	293
%	50	62	48	60	64	72	32	9	54

The percentage of cases reported differed between the two years, being less in the lighter year and varied seasonally, being less in the lighter months—indeed very low in the summer and autumn. The reporting by years is also interesting:

	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938
Rep.	5	0	71	17	173	63	341	42	839	55	42
Not	59	57	123	120	213	269	293	377	320	64	35
Total	64	57	194	137	386	332	634	419	1159	119	77
%	8	0	35	12	45	19	54	10	72	46	55

The great irregularity between the years (though apparently with an upward trend) in reporting may be noted. The two-year periodicity which might be detected in the reported cases in 1930, 1932, 1934, 1936 largely disappears when one looks at the total cases.

What the annual or seasonal variations in percentages reported in Providence may be we do not know; if the variations were very great they would affect the details of some of the calculations in the text, but apparently they would not destroy the general conclusions. It would improve the statistical discussion of the epidemiology of infectious diseases if there were more detailed studies of the factor of reporting.